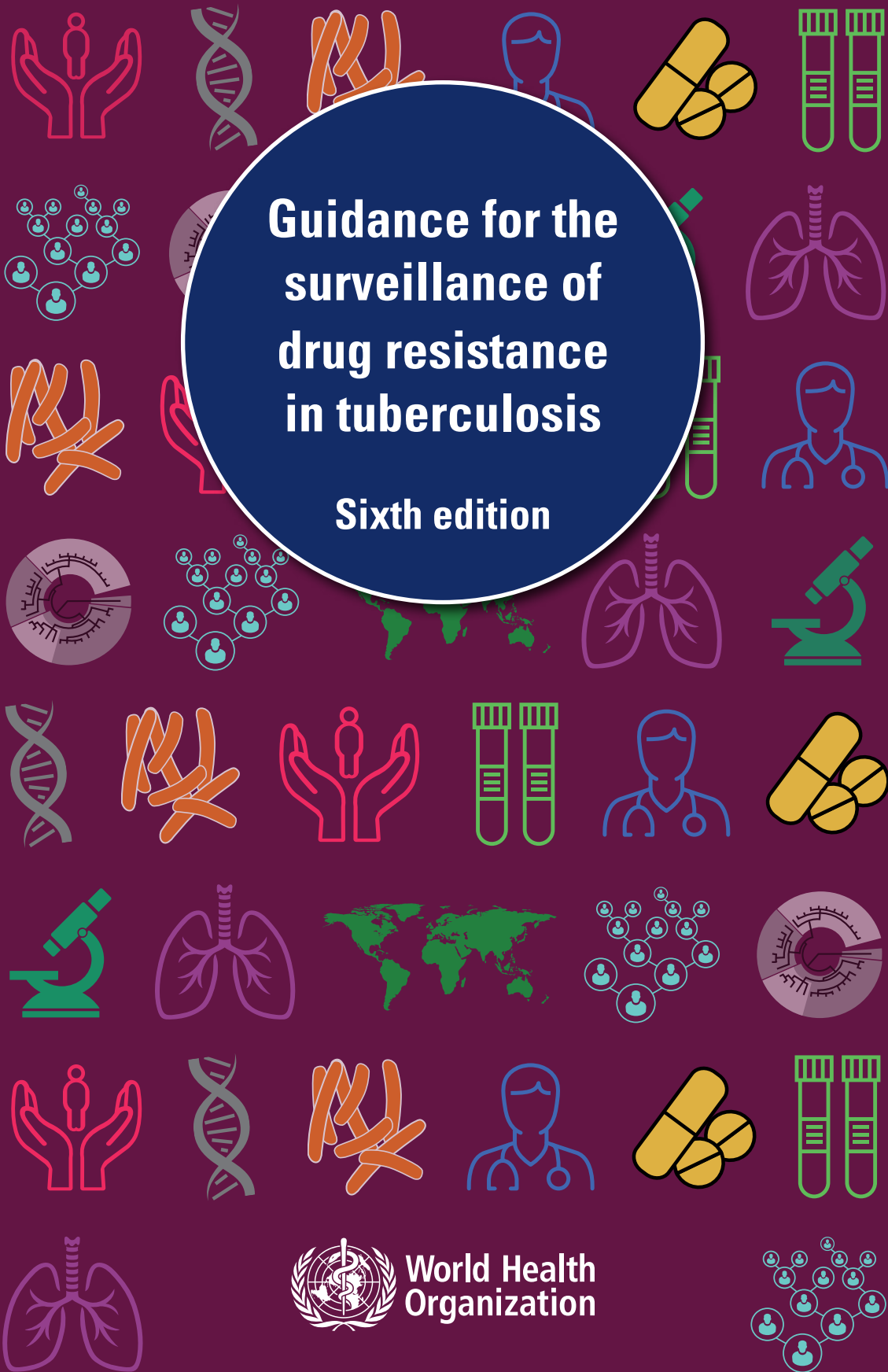


Guidance for the surveillance of drug resistance in tuberculosis

Sixth edition



World Health Organization



**Guidance for the
surveillance of
drug resistance
in tuberculosis**

Sixth edition



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Organization**

Guidance for the surveillance of drug resistance in tuberculosis, sixth edition

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Designed by Fiona Byrne.

Contents

Acknowledgements	v
Abbreviations	vi
Introduction	1
Part I Principles of anti-TB drug resistance surveillance	5
1 Mechanisms of surveillance that produce data representative of a geographically-defined population	5
1.1 Continuous surveillance systems based on routine DST	6
1.2 Periodic surveys for estimating the burden of drug resistance	7
1.3 Sentinel surveillance systems for monitoring trends over time	8
2 Standardized stratification of results by patient characteristics	8
2.1 Patient treatment history classifications	8
2.2 Age groups, sex, HIV status and other patient sociodemographic and clinical factors	9
3 Quality-assured laboratory methods for determining resistance to anti-TB drugs	10
3.1 WHO-recommended methods for DST	10
3.2 Quality assurance of DST	15
4 Ethical considerations	18
Part II Planning and conducting a survey	21
5 Survey planning	21
5.1 Survey documents and other essential documents	21
5.2 Survey governance	23
5.3 Forming a national survey coordination team	24
5.4 Setting objectives	24
5.5 Defining the laboratory algorithm	25
5.6 Development of a protocol and time schedule	27
5.7 Minimum required facilities for a survey area	28
5.8 Sampling of cases	29
5.9 Budgeting	35
5.10 Training	35
5.11 Laboratory network preparedness	36
5.12 Pilot study	37

6	Survey implementation	38
6.1	Inclusion and exclusion criteria	38
6.2	Patient enrolment	38
6.3	Sample collection, storage and transport	41
6.4	Monitoring and evaluation	44
7	Data management, analysis and dissemination of survey results	45
7.1	Data management	45
7.2	Data analysis	47
7.3	Dissemination of survey findings, and policy and practice implications	49
	References	52
	Annexes	56
Annex 1	– Examples of survey algorithm designs	56
Annex 2	– Guide for developing a survey protocol	58
Annex 3	– Guide for survey participant information	63
Annex 4	– Example of a flowchart of enrolled patients	64
Annex 5	– Tables for summarizing main results	65
Annex 6	– Template for survey budget	67
Annex 7	– Template for a case report form	69
Annex 8	– Storage of sputum samples and culture isolates	71
Annex 9	– Safe shipment of specimens	74
	Part I – Shipping of culture isolates and sputum samples	74
	Part II – Inactivation and shipment of non-infectious specimens for molecular testing	76
Annex 10	– Template for assessment of survey preparedness and monitoring	80
Annex 11	– Template for assessment of the preparedness and monitoring of the central reference laboratory	83
Annex 12	– Template for on-site assessment of the preparedness and monitoring of health facilities	88
Annex 13	– Template for remote monitoring of health facilities	91
Annex 14	– Examples of quality and progress indicators	92

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Abbreviations

CPC	cetylpyridinium chloride
CTM	capture, tracking and management
DHIS2	District Health Information Software 2
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
FIND	Foundation for Innovative New Diagnostics
GLI	Global Laboratory Initiative
Hr-TB	rifampicin-susceptible, isoniazid-resistant tuberculosis
IATA	International Air Transport Association
LPA	line probe assay
MAR	missing at random
RR-TB	rifampicin-resistant tuberculosis
MDR-TB	multidrug-resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
MTB	<i>Mycobacterium tuberculosis</i>
MoU	memorandum of understanding
MTA	material transfer agreement
NGS	next-generation sequencing
PPS	probability proportional to size
RR	rifampicin-resistant
RRDR	rifampicin resistance determining region
SOP	standard operating procedure
SRL	Supranational Reference Laboratory
TAT	turnaround time
TB	tuberculosis
WGS	whole genome sequencing
WHO	World Health Organization
XDR-TB	extensively drug-resistant tuberculosis

Introduction

This sixth edition of the *Guidance for the surveillance of drug resistance in tuberculosis* (TB) is an updated version of earlier editions published between 1994 and 2015 (1–5). Accurate diagnosis and treatment of TB should be available and accessible to all who need it, in line with the quest of the World Health Organization (WHO) to achieve universal health coverage, and to avert deaths from a preventable, treatable and curable disease. In 2014–2015, all WHO Member States committed to ending the TB epidemic by 2030 through the adoption of WHO’s End TB Strategy and the United Nations Sustainable Development Goals (SDGs) (6,7). This guidance document supports their call for improved access to diagnostic testing for TB, including universal drug susceptibility testing (DST). Furthermore, it contributes to the 2019 World Health Assembly resolution (WHA72.5) for strengthened efforts to combat antimicrobial resistance (8), with an acknowledgement of its critical importance to TB.

This updated guidance incorporates experience gained from 25 years of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance (hereafter referred to as the Global Project), a project initiated by WHO and the International Union Against Tuberculosis and Lung Disease (The Union), supported by a global network of Supranational TB Reference Laboratories (SRLs) (9). This is the oldest and largest project for the surveillance of antimicrobial drug resistance in the world. The Global Project has served as a common platform for country, regional and global level evaluation of the magnitude and trends in anti-TB drug resistance. It has quantified the global burden of rifampicin-resistant (RR) TB, multidrug-resistant (MDR) TB¹ and of extensively drug-resistant (XDR) TB². More importantly, it has assisted countries in planning the scale-up of the management of drug-resistant TB by providing essential data on national burden and drug resistance patterns.

Since its launch in 1994, the Global Project has collected and analysed data on anti-TB drug resistance from national surveillance systems and periodic surveys from 169 countries, which together account for 99% of the world’s estimated TB patients (10). Drug resistance surveillance data are published annually within the WHO Global Tuberculosis Report.

The aim of this document is to assist national TB programmes in developing the strongest possible mechanisms of surveillance, starting from periodic country-specific surveys of sampled patients. The ultimate goal is to establish continuous surveillance systems based on routine DST. These guidelines promote certain standardized criteria for surveillance to ensure that results are comparable within and between countries over time. The target audience of this document is national TB programmes and, in particular, the coordination team for surveillance ideally composed of the programme manager, a laboratory specialist, a logistician, and an epidemiologist/statistician.

1 MDR-TB: defined as *Mycobacterium tuberculosis* with resistance to rifampicin and isoniazid.

2 XDR-TB: from 2021, defined as *Mycobacterium tuberculosis* with resistance to rifampicin (RR-TB), plus resistance to a fluoroquinolone and at least one Group A drug recommended for the treatment of RR-TB.

This document is divided into two parts. Part I describes the principles of the Global Project that should be considered fundamental to routine continuous surveillance and periodic surveys, and the requirements to transition from the former to the latter. Part II describes the steps needed to plan and implement a survey to determine the burden of drug resistance, and to manage and interpret the data collected.

Changes from previous editions

Readers familiar with the 2015 edition of the *Guidelines for surveillance of drug resistance in tuberculosis* will notice the following updates in the current edition:

- To facilitate development of a comprehensive survey protocol, a guide is provided in Annex 2 as well as a list of other recommended survey documents to facilitate planning and implementation and ensure data of high quality (section 5.1).
- The central role of molecular technologies in continuous surveillance and surveys is further highlighted, either used alone or as a screening tool prior to culture-based methods (section 3.1). Advantages and limitations are presented for different tests, including Xpert® MTB/RIF, Xpert Ultra, Truenat MTB-RIF Dx and line probe assays (LPA). Examples of different diagnostic testing algorithms are given (section 5.5 and Annex 1), which should be tailored to the objectives of the survey and the available resources and capacity.
- Next-generation sequencing (whole genome sequencing and targeted gene sequencing) is introduced as a cost-effective and comprehensive tool for DST, as well as offering additional valuable epidemiological information (section 3.1).
- More comprehensive information is provided for the appropriate collection, storage and transport of samples and specimens, to ensure that the required tests can be performed on high-quality material in a safe manner. This includes both infectious (sputum, culture isolates) and non-infectious (inactivated cultures, ethanol-preserved sputum) materials (section 6.3, Annex 8 and Annex 9).
- For cluster-based surveys, a variable cluster size sample design is now presented, in addition to the previously recommended fixed cluster size design. This may be particularly relevant in settings where health facilities have small caseloads (section 5.8).
- Detailed templates have been included to strengthen the planning and implementation of the survey at all levels, ensuring high quality results. These templates provide guidance for: assessing survey preparedness and conducting monitoring of high-level governance aspects (Annex 10); assessing preparedness and conducting monitoring of the Central Reference Laboratory (Annex 11); assessing preparedness and conducting on-site monitoring of health facilities (Annex 12); and conducting remote monitoring of health facilities (Annex 13). A list of key survey quality and progress indicators is also provided (Annex 14).

There are currently other relevant guidance under development by WHO and, when published, these will override any older information contained in this document. This may include revisions of case definitions and the reporting framework for TB (including treatment outcomes for drug-susceptible and drug-resistant TB), updates to the use of laboratory technologies already recommend by WHO, and the recommendation of new technologies by WHO for TB diagnosis and DST.

Principles of anti-TB drug resistance surveillance

1. Mechanisms of surveillance that produce data representative of a geographically-defined population

“Surveillance” means the systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary.

WHO International Health Regulations (2005)

The Global Project for Anti-TB Drug Resistance Surveillance (Global Project) was initiated in 1994 with the aim of collecting and evaluating data on anti-TB drug resistance in a systematic and ongoing manner across the world. Within the standardized methodological framework designed for the Global Project, two main approaches to surveillance can collect data on drug resistance representative of a geographically-defined population in order to allow for comparison across settings and within settings over time. These two approaches are: (i) continuous surveillance based on routine DST for most TB patients using phenotypic and/or genotypic tests, and (ii) periodic surveys of a representative sample of pulmonary TB patients.

A continuous surveillance system based on routine DST is best able to meet the criteria of systematic and ongoing. However, capacity remains insufficient in many countries and it is clear that alternative measures are needed in light of region- and country-specific characteristics and capacities. For these reasons, in many countries, periodic surveys of randomly selected pulmonary TB patients remain the basis of drug resistance surveillance.

Each country should take a long-term view of surveillance and design a system that best fits current as well as projected needs. This system should be based on capacity that is sustainable, and ideally allow the evaluation of trends over time — an inherent objective of surveillance. Countries may combine components from the two key surveillance mechanisms in order to meet specific needs and capacities while moving towards the ultimate goal of routine DST for all people with TB.

The Global Project measures the prevalence of resistance among bacteriologically confirmed episodes of TB presenting to health centres (among new and/or previously treated cases - see section 6.1: Inclusion and exclusion criteria). This is assumed to

be similar to the prevalence of resistance among people with TB who do not access care. DST can be incorporated into national surveys of TB prevalence to estimate the prevalence of resistance among people with TB in the community. However, the low number of detected resistant cases often limits the precision of these estimates.

1.1 Continuous surveillance systems based on routine DST

Establishment of continuous surveillance systems for drug-resistant TB leads to improved access to timely and appropriate treatment and care. It also offers programmatic benefits including rapid detection of outbreaks, real-time monitoring of the effectiveness of interventions and an understanding of trends. Approximately two-thirds of the countries currently reporting data to the Global Project have continuous surveillance systems with quality-assured laboratories that can provide routine DST for rifampicin for most bacteriologically confirmed cases of pulmonary TB (10). Rapid molecular diagnostic tests continue to play a central role in the ongoing expansion of testing capacity, with an increasing number of countries transitioning from periodic surveys to continuous surveillance systems. Due to inherent challenges in collecting samples from patients with extra-pulmonary TB for testing, these data are not yet captured. The prevalence of resistance among cases of extra-pulmonary TB is assumed to be similar to that among cases of pulmonary TB.

Countries should aim to test at least 80% of bacteriologically confirmed new and previously treated TB cases for rifampicin resistance. In settings where a low proportion of notified TB cases are bacteriologically confirmed, adoption of sensitive diagnostic tools and improvement of the validity of clinical diagnoses is critical. Where capacity is currently not available for systematic DST, there should be prioritization of rifampicin testing for people at risk of drug-resistant TB, or for whom morbidity and mortality of drug-resistant TB may be higher. At a minimum, systematic DST should be established among all previously treated TB cases, contacts of people with drug-resistant TB, children, and people living with HIV/AIDS.

Among people diagnosed with RR-TB, DST for fluoroquinolones is essential. A revised definition for pre-XDR-TB will be applied from 2021, referring to combined resistance to rifampicin and fluoroquinolones. Testing for other Group A drugs for the treatment of RR-TB (such as bedaquiline and linezolid) is also recommended, with XDR-TB being defined from 2021 as combined resistance to rifampicin, fluoroquinolones and at least one other Group A drug. Capacity for DST should be expanded to other critical drugs, including Group B and C drugs using phenotypic, molecular and/or sequencing methods (11).

Efforts should also be invested in expanding testing for isoniazid resistance. Testing coverage for isoniazid remains low among bacteriologically confirmed new and previously treated TB cases, with the result that an important group of people with TB who are susceptible to rifampicin but resistant to isoniazid may not be detected and, thus, may not receive the recommended fluoroquinolone-containing treatment regimen. Among isoniazid-resistant, rifampicin-susceptible (Hr-TB) patients, fluoroquinolone testing should be conducted. The current TB diagnostic pipeline (including technologies still under development as well as those under

evaluation by WHO) includes several molecular tests for the rapid testing of isoniazid and fluoroquinolone resistance. Consequently, gaps in DST for these drugs may begin to lessen in the near future.

Barriers to strengthening continuous surveillance include the absence of sample referral systems, weak laboratory capacity for testing, and inaccurate and/or incomplete clinical data which is often related to the lack of electronic recording and reporting systems. Financial resources must be appropriately allocated to build these core components of a functioning surveillance system.

Diagnostic connectivity solutions for systems with automated readers that produce results in digital format (for example GeneXpert® platform, Bactec Mycobacteria Growth Indicator Tube (Bactec™ MGIT™), line probe assays and Truelab micro PCR analyzer) facilitate real-time monitoring and evaluation, and allow assessment of the implementation of laboratory diagnostic algorithms and testing coverage. They also provide a highly cost-effective way to ensure proper functioning of a diagnostic device network and improve linkage to patient treatment and care (12). Test results can be transferred electronically to clinicians and automatically integrated into laboratory information management systems or electronic registers.

1.2 Periodic surveys for estimating the burden of drug resistance

In resource-constrained settings where capacity is still being developed for conducting routine rifampicin DST of most bacteriologically confirmed pulmonary TB cases, national surveys should be conducted to measure drug resistance among a random sample of patients which is representative of the geographically-defined population under study. When properly designed and periodically conducted, such surveys soundly estimate the resistance profile of all patients with TB in the country and can detect general trends over time. While some countries may have achieved routine DST coverage of 80% for rifampicin among cases of bacteriological confirmed pulmonary TB through continuous surveillance, testing coverage may remain suboptimal for isoniazid as well as for second-line drugs among patients with rifampicin resistance and isoniazid resistance. In these settings, a survey should also be considered.

Periodic surveys can provide much of the same critical information provided by a continuous surveillance system. However, these periodic surveys are unable to detect localized outbreaks; may produce results with margins of error that prevent meaningful analysis or determination of trends; and may be subject to biases inherent in sampling only a subset of the population. Nonetheless, conducting surveys can build and strengthen overall laboratory capacity, sample transport and referral systems, and data management expertise, as well as provide an evaluation of the accuracy of routine classification of patients according to treatment history. Surveys offer an opportunity for detailed in-depth investigations of characteristics of the TB patient population and anti-TB drug resistance patterns, using advanced methods which are not usually integrated into continuous surveillance. Application of sequencing technologies, for example, can provide valuable insights into the phylogenetics of the circulating TB strains. Surveys can also provide a platform for studying risk factors for drug resistance (see section 2.2.3: Other sociodemographic and clinical factors).

1.3 Sentinel surveillance systems for monitoring trends over time

For countries where limited resources, health care system structure, or geographical features preclude routine DST of all patients in surveillance systems, the establishment of a sentinel surveillance system may be an option for monitoring trends in drug resistance over time. Sentinel sites should ideally be drawn from a range of geographical and socioeconomic areas. They should be centres with a moderate to high TB caseload with the capacity for testing by rapid molecular methods.

A sentinel system could be a useful interim approach for countries in the process of establishing routine continuous surveillance. However, it has several important limitations. Unlike national surveys, the health facilities acting as sentinel sites are selected purposely rather than randomly, and therefore cannot be used to estimate the prevalence of drug resistance at the national level. Additionally, the data cannot be used to make inferences with respect to trends in the rest of the country. A sentinel system is therefore only recommended for countries which have high quality data from a recent survey (within the previous five years) and which are moving towards establishing national systems for continuous surveillance.

2. Standardized stratification of results by patient characteristics

2.1 Patient treatment history classifications

Careful classification of treatment history is critical to proper and accurate interpretation of surveillance data. The January 2020 update of the 2013 revision of WHO's *Definitions and reporting framework for tuberculosis (13)* defines patient registration groups using history of previous treatment. A revised edition of this document is expected in 2021, with updated case definitions for drug-susceptible and drug-resistant TB.

New case

For the purpose of surveillance, a “new case” is defined as a newly registered episode of TB in a patient who, in response to direct questioning, reports never having been treated for TB or reports having taken anti-TB drugs for less than one month; or, in countries where adequate documentation is available, for whom there is no evidence of having taken anti-TB drugs for one month or more.

Previously treated case

For the purpose of surveillance, a “previously treated case” is defined as a newly registered episode of TB in a patient who, in response to direct questioning, reports having received one month or more of anti-TB drugs in the past; or, in countries where adequate documentation is available, there is evidence of having received one month or more of anti-TB drugs.

Previously treated patients are at higher risk of having strains of TB with resistance to one or more drugs. Information about the size and composition of this population

and the patterns of resistance in subcategories of previously treated cases is important for programmatic reasons. Subcategories of previously treated cases currently include:

- Relapse patients have previously been treated for TB, were declared as “cured” or “treatment completed” at the end of their most recent course of treatment, and have been diagnosed with a recurrent episode of TB. This could either be a true relapse or a new episode of TB caused by reinfection.
- Treatment after failure patients are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment.
- Treatment after loss to follow-up patients have previously been diagnosed with TB but did not start treatment or their treatment was interrupted for at least two consecutive months.
- Other previously treated patients (also referred to as “outcome not evaluated”) are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented, and therefore may include some patients who were lost to follow-up.

2.2 Age groups, sex, HIV status and other patient sociodemographic and clinical factors

Given the large imbalance in numbers of drug-susceptible and drug-resistant patients in most surveys, it may not be possible to detect significant differences between patient groups. Although a case-control study is a more appropriate design for exploring risk factors for drug resistance, the opportunity provided by drug resistance surveys should not be overlooked. Additionally, basic patient demographic data can be used to inform imputation models which may be used for handling missing data (see section 7.2.1: Imputation of missing values).

2.2.1 Age groups and sex

Data on drug resistance stratified by age groups and sex can provide insight into risk groups and effectiveness of specific TB control activities. Furthermore, the magnitude of drug resistance among younger age groups is more likely to be indicative of recent transmission than among older age groups, which may be harbouring older infections.

2.2.2 HIV status

Depending on the nature of the HIV epidemic in a given setting, incorporation of HIV testing in anti-TB drug resistance surveillance may yield important information for the national TB programme on the relationship between HIV and drug-resistant TB. The national HIV/AIDS programme should be involved in all stages of the planning and execution of surveillance. Existing national policies on HIV testing should be followed, including the availability of counselling services, and ensuring consent and confidentiality procedures. Provider-initiated HIV testing is recommended for patients presenting with signs and symptoms suggestive of TB and whose HIV status is undocumented. For those with a documented test result, the date of testing should

be captured on the case report form (see section 6.2.1: Case report form) and retesting should be considered if a negative result is more than six months old.

2.2.3 Other sociodemographic and clinical factors

Inclusion of other patient sociodemographic and clinical information can be considered depending on the setting, the objectives of the survey and available resources. Surveys can serve as a valuable platform for studying setting-specific causes of drug resistance and possible targets for intervention.

Other sociodemographic factors that may be evaluated include: contact with a patient with drug-resistant TB; type of health facility (for example public or private sector); patient residence (for example urban or rural); socioeconomic status; education level; employment status (and if employed, type of employment); country of birth; and history of migration or mobility. Clinical factors may include: malnutrition; diabetes; alcohol abuse; injecting drug use; smoking; and previous exposure to TB preventive therapy. For previously treated patients, additional information could include: date and type of previous treatment and treatment supervision; composition of treatment regimens; and source of drugs used. It should be noted that multiple risk factors for acquisition, amplification and transmission of drug resistance may be present simultaneously in a given setting.

For examples of how to design questions to measure social determinants, see Lönnroth et al (14) or Annex 5 of the 2011 WHO publication, *Tuberculosis prevalence surveys: a handbook* (15) (revised edition expected in 2021). The examples provided may require modification based on local conditions and the population under study.

3 Quality-assured laboratory methods for determining resistance to anti-tb drugs

The establishment of quality-assured laboratory techniques using WHO-recommended methods is essential for reliable surveillance of drug resistance. The introduction of rapid molecular technologies into diagnostic algorithms for the identification of *M. tuberculosis* complex and DST should be prioritized (16,17). No single test has an accuracy of 100%, and each method has advantages and disadvantages to be considered when designing a laboratory algorithm. Due to the dynamic nature of research and development, new technologies other than those described below may have been recommended by WHO since the publication of this document. These could also be incorporated into surveillance activities.

3.1 WHO-recommended methods for DST

In addition to conventional phenotypic DST, rapid methods for DST are available using diagnostics that can be feasibly implemented into a variety of settings worldwide. These facilitate clinical decision-making to ensure timely initiation of an appropriate treatment regimen based on a patient's drug resistance profile, as well as enhanced capacity for surveillance. Comprehensive guidance on rapid diagnostics for

detection of drug-resistant TB is provided in Module 3: diagnosis - rapid diagnostics for tuberculosis detection in the *WHO consolidated guidelines on tuberculosis* (16). Examples of diagnostic algorithms that could be incorporated into routine continuous surveillance are provided in Module 3: diagnosis - rapid diagnostics for tuberculosis detection of the *WHO operational handbook on tuberculosis* (17).

3.1.1 Phenotypic DST

Culture and identification of *M. tuberculosis* complex must be performed according to WHO recommendations (18–20). Culture-based phenotypic DST methods consist of testing of a culture of *M. tuberculosis* complex at critical concentrations of anti-TB agents to determine susceptibility or resistance. Phenotypic methods are time-consuming and require sophisticated laboratory infrastructure, qualified staff and strict quality assurance mechanisms (21). For these reasons, phenotypic DST is being progressively replaced by molecular-based DST for core first-line and second-line drugs. The currently recommended critical concentrations for testing are given in WHO's *Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis* (2018) and WHO's *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis* (2018) (21,22). Revised critical concentrations for some drugs may be issued after publication of this document, and these will override those concentrations defined in earlier guidance documents.

BIOSAFETY MEASURES

All procedures involving the handling of specimens for culture and DST should be carried out in a high-risk TB laboratory, as defined in WHO's *Tuberculosis laboratory biosafety manual* (19) and in the Global Laboratory Initiative's (GLI) *Tuberculosis laboratory safety: the handbook, global edition* (20). Particular care needs to be taken when bottles are being opened, closed or shaken and when materials are being centrifuged, all of which may lead to the production of infectious aerosols. The transportation of TB cultures presents important risks in the event of accidents or container breakage. It is therefore essential that the exchange of strains between the Central Reference Laboratory and the SRL is carried out according to the regulations outlined in Annex 9.

Phenotypic DST is currently not recommended for ethambutol, thioamides (prothionamide, ethionamide), cycloserine, terizidone, and other Group C medicines (p-aminosalicylic acid, imipenem-cilastatin, meropenem) due to either inconsistent results or as yet undefined critical concentrations for testing, although this may change in the future (21,22). Work is underway in defining standardized DST for pretomanid.

Phenotypic DST using solid media

Conventional phenotypic methods using solid media remain common in many settings, using egg-based (such as Löwenstein Jensen or Ogawa) or agar-based (such as Middlebrook 7H10/7H11) media. Methodology is well described elsewhere. The proportion method is recommended for DST in solid media (22).

Advantages: DST in solid media is relatively inexpensive and highly standardized for testing susceptibility to many drugs.

Limitations: Up to eight weeks are required to produce a definitive confirmation of TB, and another six weeks are required for DST results. Results are unreliable for pyrazinamide and clofazimine. The critical concentrations for testing of new and repurposed drugs have not been established for Löwenstein Jensen.

Phenotypic DST using liquid media

DST using the BACTEC MGIT system is the preferred method for performing DST for many anti-TB agents, given the standardization of the MGIT media and instrument (22).

Advantages: Confirmation of TB can usually be obtained within two to three weeks, with DST results available in an additional one to two weeks. Liquid culture methods can be used for susceptibility testing for first-line and second-line drugs, as well as new (bedaquiline and delamanid) and repurposed (clofazimine and linezolid) drugs.

Limitations: The disadvantages of the liquid culture method and DST include a relatively high cost for equipment and consumables; the need for appropriate laboratory infrastructure (particularly biosafety precautions); and the longer turnaround time for providing results compared to molecular-based DST methods. MGIT is the only WHO-recommended phenotypic method for pyrazinamide susceptibility testing, although results can be inconsistent. Sequencing is preferred to phenotypic DST for pyrazinamide.

3.1.2 Nucleic Acid Amplification Tests

Nucleic Acid Amplification Tests are molecular methods for the detection of drug-resistant TB. They have considerable advantages over phenotypic DST methods for scaling up surveillance of drug-resistant TB: rapid diagnosis, standardized testing, the potential for high through-put, and fewer requirements for laboratory biosafety. Molecular methods detect deoxyribonucleic acid (DNA) from both viable and non-viable organisms and from mutations associated with drug resistance. Detailed information on the tests described below is available from the *WHO consolidated guidelines* and the *WHO operational handbook on rapid diagnostics for tuberculosis detection* (16,17). The TB diagnostic pipeline includes molecular technologies still under development as well as others under currently evaluation by WHO, and these could be incorporated into future surveillance activities.

Xpert MTB/RIF and Xpert MTB/RIF Ultra

Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is a fully automated real-time PCR assay that integrates sputum processing, DNA extraction and amplification, semi-quantitative diagnosis of *M. tuberculosis* and detection of rifampicin resistance. This cartridge-based system detects common mutations in the Rifampicin Resistance Determining Region (RRDR) of the *rpoB* gene (between *M. tuberculosis* codon positions 428 and 452) in both smear-positive and smear-negative sputum specimens, with Xpert MTB/RIF Ultra showing improved sensitivity for detection of

M. tuberculosis complex from smear-negative cases and mixed infections. An Xpert MTB/RIF training package is available online from the Global Laboratory Initiative as well as a guide for implementing quality assurance systems for Xpert MTB/RIF.

Advantages: The test can be used at the peripheral level, as facilities offering Xpert MTB/RIF or Xpert MTB/RIF Ultra require biosafety requirements similar to those for direct sputum-smear microscopy. Test results are obtained in less than two hours. The test can be conducted directly on sputum or concentrated sediments. Multiple samples can be tested in parallel.

Limitations: In settings where circulating *M. tuberculosis* strains display mutations or other nucleotide variants outside of the region of the *rpoB* gene that is targeted by the assay, patients with resistance may be diagnosed as susceptible to rifampicin.

Truenat MTB-RIF Dx

Truenat MTB-RIF Dx (Molbio Diagnostics, Goa, India) is a chip-based real time PCR test that detects the presence of common mutations in the RRDR of the *rpoB* gene (between codon positions 428 and 452). The test is used as reflex test on samples that are already detected as positive for *M. tuberculosis* complex by Truenat® MTB or MTB Plus, which are semiquantitative real-time PCR tests. Interim data from a multi-site, field evaluation study has shown similar accuracy of Truenat MTB-Rif Dx to WHO-approved commercial line probe assays for rifampicin resistance detection.

Advantages: Test results are available in two hours. The Truenat system requires only a minimal fresh sputum sample volume. The system is designed to enable decentralization and near patient diagnosis of *M. tuberculosis* complex.

Limitations: The test is not fully automated. Therefore, it is important to follow good laboratory practices and strictly adhere to the test procedures to minimize the risk of contamination by PCR amplification products. In addition, mutations or other nucleotide variants outside of the region of the *rpoB* gene that is targeted by the assay will be missed and patients will be misdiagnosed as susceptible to rifampicin.

Line probe assays

Line probe assays (LPAs) detect *M. tuberculosis* complex and the most common mutations that confer resistance to anti-TB drugs. Commercial LPAs for first-line DST, such as the GenoType MTBDRplus version 2 assay (Hain Lifescience, Nehren, Germany) or Nipro NTM+MDRTB detection kit 2 (Tokyo, Japan), include *rpoB* probes to detect rifampicin resistance, *katG* probes to detect mutations associated with high-level isoniazid resistance, and *inhA* probes to detect mutations usually associated with low-level isoniazid resistance. LPAs for detection of second-line drugs, such as the GenoType MTBDRsl version 2 assay (Hain Lifescience, Nehren, Germany), incorporate probes to detect mutations within genes which are associated with resistance to either fluoroquinolones (*gyrA* and *gyrB* genes) or second-line injectable drugs (*rrs* gene and the *eis* promoter region).

Advantages: LPAs can be performed on culture or directly on sputum, although the rate of indeterminate results is higher for smear-negative sputum samples. For

patients that have been diagnosed with rifampicin and/or isoniazid resistance, second-line LPAs can provide a rapid DST for fluoroquinolones to guide treatment options.

Limitations: The sensitivity of LPAs to detect resistance to isoniazid, fluoroquinolones, aminoglycosides and cyclic peptides is approximately 85% and thus lower than that of culture methods. This is because some mutations conferring resistance are outside the regions covered by the test. Although some probes detect specific mutations, others are only inferred by lack of probe binding. The test requires multiple pieces of equipment, adequate infrastructure and more specialized expertise than Xpert MTB/RIF, Xpert MTB/RIF Ultra or Truenat MTB-RIF Dx.

3.1.3 Next-Generation Sequencing

Whole Genome and Targeted Gene Sequencing

Next-generation sequencing (NGS) refers to high-throughput sequencing technologies used to determine the nucleotide sequence of a whole genome (whole genome sequencing, WGS) or part of a genome (targeted NGS) in a single biochemical reaction volume. NGS is performed by non-Sanger-based sequencing methods that are capable of simultaneously sequencing multiple DNA fragments in parallel, which are then pieced together by *de novo* assembly and mapped to a known reference genome using bioinformatics tools.

Advantages: NGS overcomes many of the significant challenges associated with conventional phenotypic testing as well as the limitations of other less comprehensive molecular tests (23). Depending on local costs for personnel, reagents and other laboratory requirements, it may be more cost-effective to perform NGS than phenotypic DST for multiple drugs. It is currently the only approach that has the ability to interrogate hundreds of genome-wide targets in parallel and simultaneously test for resistance to multiple first and second-line anti-TB drugs. As a result, it can detect rare mutations that are typically missed by rapid molecular assays. Sequencing also allows species identification, genotyping, and detection of mixed populations and heteroresistance in a sample (24). Targeted NGS generates sequence data at specific genetic loci. The definition of the most important loci will continue to evolve as more evidence becomes available. All-in-one targeted NGS assays are commercially available that can provide comprehensive DST without the need for culture. For example, the Deeplex®-MycTB assay (GenoScreen, Lille, France) allows genotyping of mycobacterial species and resistance prediction for up to 15 drugs based on sequencing of up to 18 targets associated with resistance (Deeplex®-MycTB assay [GenoScreen, Lille, France]). Targeted NGS assays are highly flexible and can be modified to include additional genetic loci as evidence on genetic markers of resistance for existing and new drugs continues to evolve.

WGS has an advantage over targeted NGS as it can provide information across the entire genome. This is useful for identifying transmission chains, disease clusters and outbreaks, as well as for identifying novel resistance mechanisms for both existing and new drugs. WGS can currently only be performed reliably and cost-effectively using culture isolates, due to the need for large amounts of good quality DNA, whilst targeted NGS can be applied directly on preserved sputum samples.

In terms of implementation, infrastructure and skill requirements are similar for both WGS and targeted NGS. The choice of technology will depend on considerations such as intended settings, applications, and cost constraints. The upcoming publication by WHO and the Foundation for Innovative New Diagnostics (FIND), *Practical considerations for implementing next-generation sequencing for drug resistance surveillance in national TB programmes*, will provide practical guidance to national TB programmes and laboratories to plan and implement NGS-based approaches for the detection and characterization of the *M. tuberculosis* complex, with an emphasis on detection of mutations associated with drug resistance and molecular epidemiology for surveillance purposes (25).

Limitations: NGS currently requires advanced laboratory infrastructure and molecular expertise. The global uptake of NGS faces challenges in programmatic integration into existing laboratory workflows due to perceived cost and technical barriers, including rigorous laboratory and data management infrastructure requirements, technical complexity of NGS workflows and need for expert guidance in bioinformatics analysis and data interpretation, and the lack of readily-available solutions for data analysis and data storage (26). Understanding of the genetic basis of phenotypic drug resistance continues to evolve. Efforts to produce a global clinical knowledge base of genetic determinants of phenotypic resistance are ongoing and will help to ensure standardized and accurate interpretation of NGS data. Currently, phenotypic DST should continue to be conducted for new and repurposed medicines, ideally in parallel with NGS.

3.2 Quality assurance of DST

A comprehensive laboratory quality assurance system is essential to ensure that the results of DST are accurate, reliable and timely. The key components of a comprehensive quality assurance programme for DST include the use of standard operating procedures (SOPs) in line with WHO recommendations, internal quality controls, external quality assessment (including proficiency testing and regular on-site supervision) and quality indicator monitoring.

Quality control should be performed on new batches of test kits and reagents (new lot testing) to ensure that the testing material is fit for use and that the transport and storage conditions have not affected the assay performance. This usually consists of testing a sample or a set of samples using the new lot and comparing the results to those obtained with assays or reagents with known performance. The results from quality control testing must be recorded and unexpected results investigated. Trends over time should be monitored. Quality control should also be performed by new personnel prior to testing clinical specimens to assess their competency.

3.2.1 Internal quality control

Internal quality control ensures that the information generated by the testing site is accurate, reliable and reproducible. Quality control involves the testing of control materials at the same time and in the same manner as patient specimens in order to monitor the accuracy and precision of a test.

Culture-based DST

As part of internal quality control for phenotypic DST, the quality of the culture medium should be controlled with each batch of isolates tested. Drugs added to the medium must be pure substances obtained from a reputable source and properly stored. Drug dilutions and the addition of these to the medium should be performed in accordance with accepted SOPs.

It is important to perform internal quality control of culture-based DST periodically. The best practice is to run a fully susceptible quality control strain with each batch being tested. The H37Rv *M. tuberculosis* strain (American Type Culture Collection -ATCC- 27294) is suitable because it is susceptible to all anti-TB agents. It can be useful to include a resistant strain with each batch for the detection of major errors in the preparation of drug stock solutions (22), which can be included in a proficiency testing panel from the SRL. Internal quality control procedures need to be applied to all new batches of drug-free and drug-containing media, and results should always be validated by a supervisor who will ensure that all strains with doubtful results are re-tested.

Molecular-based DST

Common quality control substances for molecular-based DST include strains of *M. tuberculosis* complex with well-characterized drug resistance profiles (strains carrying a known set of mutations associated with drug resistance), DNA extracts from these strains, or previously characterized clinical samples. Negative quality control samples include water or solutions used to extract or to amplify the bacterial DNA.

Internal quality controls are often built into commercial devices. Each Xpert MTB/RIF and Xpert MTB/RIF Ultra cartridge contains a Sample Processing Control and a Probe Check Control. The GenoType MTBDR line probe assays include Conjugate Controls and Amplification Controls. Truenat MTB-RIF Dx assay has no integrated internal controls.

Sequencing-based DST

As most Central Reference Laboratories do not yet have capacity to perform sequencing, this is mainly performed at SRL level. In general, given the multiple steps of the NGS workflow and the lack of commercially available end-to-end solutions for WGS, quality checks should be performed after each of the main steps of the process, including assessment of the sample (quality and quantity of *M. tuberculosis* in the clinical isolate or specimen), DNA extraction (quality and quantity of DNA obtained), library preparation, sequencing, sequence assembly and analysis, and variant calling (23,25). Examples of internal quality controls are the use of a negative control (e.g. water-only sample) in each batch of samples undergoing DNA extraction, and the use of genomic DNA from a reference strain, such as H37Rv or *M. bovis* BCG, as a control for library preparation and sequencing (25). Further details on NGS quality assurance, control and assessment will be published by WHO and FIND in the upcoming guide *Practical considerations for implementing next-generation sequencing for drug resistance surveillance in national TB programmes* (25).

3.2.2 External quality assessment and the role of the SRL Network

External quality assessment has several components: proficiency testing, retesting of strains, and onsite evaluations of laboratories, all of which are conducted in cooperation with an external partner laboratory.

The SRL network plays a critical role in capacity strengthening of laboratories worldwide and is fundamental in the external quality assessment activities that ensure the accuracy of national surveillance of drug resistance. At the time of publication of this document, there were 32 SRLs and four National Centres of Excellence in the network (9). See full list at: <https://sites.google.com/site/srtblaboratories/list>.

SRLs maintain a high level of quality by participating in annual intra-network proficiency testing for DST. The SRLs establish a consensus on the susceptibilities of selected strains to a range of drugs used for the treatment of TB. The panels of strains are subsequently used to assess the proficiency of Central Reference Laboratories and any subnational reference laboratories that provide DST results for surveillance systems and drug resistance surveys. SRLs can also provide onsite evaluations and training and supervision as necessary.

The assessment of a Central Reference Laboratory's accuracy at phenotypic DST by the SRL requires an exchange of *M. tuberculosis* strains: from the SRL to the Central Reference Laboratory, and from the Central Reference Laboratory to the SRL.

From the SRL to the Central Reference Laboratory (proficiency testing): A Central Reference Laboratory should annually receive a panel of pre-coded strains from a partner SRL to be tested for susceptibility to first- and second-line drugs, and, if applicable, to new and re-purposed drugs. The test results of the Central Reference Laboratory should be compared with the pre-coded results of the judicial consensus of the SRL, which can be considered a "gold standard".

From the Central Reference Laboratory to the SRL (quality assessment of results, also known as "retesting"): In order to assure the quality of phenotypic DST, a sample of strains isolated during surveillance or surveys should be sent to a partner SRL to be retested. The results should be compared for agreement with respect to each drug. National and international rules and regulations (see Annex 9) and turnaround times for shipment to the SRL must be considered for planning purposes.

The proficiency of the Central Reference Laboratory in conducting molecular-based DST can be assessed on the same strains used to assess phenotypic DST proficiency. The Central Reference Laboratory can choose the genotypic testing methods, which ideally should be those routinely in use (for example Xpert MTB/RIF, LPA, sequencing), for as many drugs as possible.

A formal proficiency testing program coordinated by SRLs for WGS does not yet exist. A pilot programme has been developed in Europe within the European TB National Reference Laboratories Network (25). The proficiency panel includes a set of ten inactivated isolates of *M. tuberculosis* complex as well as a set of five raw sequence

data (FASTQ files). Participating laboratories evaluate the presence of mutations associated with resistance to anti-TB drug and the genotype of the strains, and also perform a relatedness analysis to assess the similarity of the strains. Certificates are issued to laboratories that attain a minimum pre-defined score (25).

3.2.3 Monitoring and analysis of quality indicators

Routine monitoring of quality (or performance) indicators is the most effective way to ensure the accuracy of the laboratory results and identify areas for improvement. Quality indicators should be collected and analysed on a monthly basis for all the different tests carried out in the laboratory. They should be routinely reviewed by the laboratory manager and linked to corrective actions if unexpected results or trends are observed. A standard set of quality indicators should be used by all sites participating in the survey; a list of quality and progress indicators proposed for use during surveys is given in Annex 14.

In addition to general laboratory quality indicators (such as number of tests performed, service interruptions, turnaround time, external quality assurance and quality control results) which apply to all technologies and should be disaggregated by test type, there are also test-specific quality indicators. When applicable, test-specific quality indicators should be disaggregated according to the type of sample tested (such as clinical specimens or culture isolates), and according to the specific population group tested (such as new versus previously treated cases).

Examples of common quality indicators for phenotypic and molecular-based DST are given in Annex 14. A more comprehensive list, including relevant targets, can be found in the GLI's *Practical guide to TB laboratory strengthening* (27) and for Xpert MTB/RIF, in the *Practical guide to implementing a quality assurance system for Xpert MTB/RIF Testing* (28). A comprehensive list of quality indicators for sequencing-based DST is currently under development and will be published by WHO and FIND in the upcoming guide *Practical considerations for implementing next-generation sequencing for drug resistance surveillance in national TB programmes* (25).

4. Ethical considerations

Countries have an obligation to develop appropriate, effective mechanisms to ensure ethical surveillance through continuous systems or periodic surveys (29). The information obtained should be used to inform the development of the health system, including priority setting and strengthening diagnostic and treatment services. Countries consequently have an obligation to ensure that the data collected are timely, reliable and valid. Those responsible for conducting surveillance and surveys should identify, evaluate, minimize and disclose risks for harm before implementing these activities. Ongoing monitoring for harm is essential and appropriate action should be taken if such an event occurs.

WHO's *Guidelines on ethical issues in public health surveillance* (29) provide guidance for ethical implementation of these activities, with special consideration of the principles of common good, equity, respect for persons and effective governance.

The overarching goal of public health activities is to promote population health, but the rights, freedom, privacy and confidentiality of individual patients must be respected in planning and implementing a surveillance system or a survey. Individuals or groups who are particularly susceptible to disease, harm or injustice may be vulnerable to stigmatization, exploitation or discrimination. Special considerations may be required to avoid imposing any unnecessary additional burden on these groups during surveillance activities (29).

In order to ensure adherence to ethical standards, staff should be trained on all applicable ethical principles and processes. Survey protocols and new surveillance systems in the planning stage should be reviewed by ethics committees or institutional review boards. Such reviews should include due consideration of the below-mentioned key concepts for the ethical conduct of surveillance (29–32). Detailed guidance applicable to surveys can be found in WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys* (33).

Confidentiality

In general, sensitive patient information should be kept confidential unless its disclosure has been authorized by the person concerned. However, it may be permissible to disclose some medical information without patient consent for legitimate public health purposes (for example, mandatory reporting of certain infectious diseases). In practice, personal data should be shared only where strictly necessary for the functioning of the surveillance system and/or for the promotion of crucial public health goals. Unjustified disclosure of personal information would not only violate the patient's privacy, but could also foster stigma and discrimination.

Informed consent

In the course of a survey, informed consent or assent (used to express willingness to participate in research by persons who are by definition too young to give informed consent but old enough to understand the proposed research in general) should be obtained from individuals who have the capacity to make their own decisions. Children or vulnerable participants should be provided with information that is age-appropriate and should provide their assent in accordance with the national laws of the country. A legally authorized representative must sign and date the consent in the case of a minor or other vulnerable participant who does not have the capacity and competency to make his/her own choices. If a participant (or his/her legally authorized representative, where applicable) is unable to read or write, the process must be witnessed by an impartial literate adult. The witness should sign and personally date the consent form (33).

In contrast to the usual practice in medical research, individual informed consent is not always feasible or appropriate for continuous surveillance, especially when obtaining information from an entire population is essential to achieving critical public health objectives. Nonetheless, whenever feasible, public health practitioners should strive to obtain consent from the subjects of surveillance. Even when obtaining individual consent is deemed unfeasible or inappropriate, individuals and/or communities should be informed about the nature and purposes of the surveillance to

the extent this is possible. Surveillance systems should report results back to clinicians and consenting individuals, and those with drug-resistant TB should be referred for appropriate treatment and care. Informed consent or assent should be sufficiently detailed to describe the use and storage of an individual's data and specimens, the clinical implications of DST results, and the confidentiality arrangements in place. Ample time should be given to individuals to decide on their voluntary participation. Participants should be informed of their rights, including their right to opt-out or withdraw at any time from the survey. The following are examples of the types of information that may be addressed during the consent process:

- electronic record keeping and timeline of record storage;
- access to clinical samples and identifiable electronic patient records by clinicians, surveillance officers or others;
- storage of specimens beyond the duration of the survey and timeline of specimen storage;
- specimen transfer agreements and international shipment of specimens for further testing;
- foreseeable uses of samples and intended goal of such uses, including use of specimens in defined or as yet undefined studies;
- reporting and dissemination of results;
- HIV testing and/or use of previous HIV test results;
- access to treatment and clinical management; and
- reimbursements for relevant expenses where appropriate.

A guide for developing a structured participant information sheet for surveys is given in Annex 3. A generic checklist of contents to ensure all key components are included in both the patient information sheet and the consent assent form is provided in WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys* (33).

Access to treatment

Surveillance of drug resistance in TB raises a particular ethical dilemma where there is limited capacity to properly treat patients identified with drug-resistant strains. Provisions must therefore be in place ahead of a survey or surveillance programme to facilitate communicating results back to participants and to ensure that all people with drug-resistant TB have access to appropriate treatment and care in line with the most recent WHO guidelines.

Planning and conducting a survey

5. Survey planning

Conducting a drug resistance survey that will provide accurate, precise, complete and timely results requires significant planning. In order to obtain data that are representative of the geographically-defined population under study, the process for selecting patients must be carefully designed. Measures must be in place to ensure that the data collected are properly categorized, checked and validated, and that the DST is quality-assured. This requires comprehensive and accurate planning of logistics, including pre-survey budgeting of all planned expenses.

Good clinical practice and good data management practices guide the conduct of clinical trials, and these are also relevant to drug resistance surveys. Where feasible, these approaches should be integrated into the planning, implementation, analysis and dissemination of surveys. This will protect the rights, safety and well-being of survey participants, as well as ensure credibility of the data collected and results. For more information, refer to WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys* (33).

5.1 Survey documents and other essential documents

Tools should be developed in advance of implementing a survey to help manage anticipated risks and challenges and to ensure conformance with agreed procedures and good practice. The relevant national and/or institutional ethics committees must review and approve the protocol, any survey documents and materials used for the enrolment of participants, and any subsequent amendments to these documents. At a minimum, the following documentation should be available in advance:

- Survey protocol: A document outlining the design, objectives, methodology and overall organisation of the work to be carried out, which provides a guide for the survey as a whole. A protocol guide or checklist can be used to ensure that all required elements of a survey protocol are covered in the final document (see Annex 2).
- Standard operating procedures (SOPs): Step-by-step instructions to help survey staff carry out specific survey operations and procedures outlined in the protocol.

- Survey communications plan: A document detailing the survey governance structure; the roles and responsibilities of all organizations involved with the survey; the composition of the scientific advisory committee (where applicable) or technical assistance, the survey coordination team and the survey field team; the communication mode (meetings, reports, other) and schedule between all parties; and the escalation pathways to raise emerging and/or unresolved issues identified during the survey. The communication plan includes a RACI matrix whereby the role and responsibility of each stakeholder is defined for each survey task or activity, a survey team contact list, and an organigram showing the governance structure and reporting lines for the survey.
- Survey quality plan: A general document outlining the quality management system and the quality assurance and control measures that will be in place to satisfy the quality requirements for the survey. The plan includes an index of survey SOPs; an outline of procedures related to the lifecycle management of all SOPs; an overview of how the project, the data and the survey documentation will be managed (for instance, with defined timelines, milestones and deliverables to assess progress); an overview of induction, training and competency assessments for personnel; an overview of selection criteria for monitor and auditor staff; and roles and responsibilities of staff in relation to quality, training and handling of protocol deviations and corrective actions.
- Delegation log: A log listing all survey staff and what responsibilities they are expected to undertake. The delegation log is also used to verify that experience and qualifications of people holding those roles are adequate and that staff have received appropriate training for the assigned tasks.
- Survey monitoring plan: A document detailing the survey monitoring schedule and strategy as well as the tools (such as checklists and report templates) that will be used to document the training and preparedness of sites to start the survey, and those that will be used to monitor survey sites and report on the findings. Examples of tools include the template for assessment of survey preparedness and monitoring (Annex 10), the template for assessment of the preparedness and monitoring of the Central Reference Laboratory (Annex 11), the template for on-site assessment of the preparedness and monitoring of health facilities (Annex 12) and the template for remote monitoring of health facilities (Annex 13).
- Risk management plan: A document detailing proactive actions to identify, assess, monitor, report and respond to risks, including risks to sample quality, data integrity and protection of survey patient rights, safety and well-being. A risk assessment matrix – used for the overall process of risk identification and evaluation of the severity – should be included in the risk management plan.

Additional survey documents that should be finalized before the start of the survey include:

- participant information sheet (Annex 3) and informed consent and assent forms;
- data collection tools (e.g. patient questionnaires; see Annex 7);
- data management and analysis plans;

- training packages and training logs to monitor induction and continued training of staff;
- dissemination strategy for results;
- policies addressing ownership, access to, and re-use of data and samples;
- financial and technical agreements, including Material Transfer Agreements between laboratories¹; and
- ethical approval of survey documents and documented approval of any subsequent amendments.

Templates for developing these plans, checklists, logs, and forms to support survey management, supervision and monitoring are available from WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys* (33). Plans may be produced as stand-alone documents or be integrated within the survey protocol (see Annex 2). Additional tools available include, among others: a participant enrolment log; an incident log (to document any incidents of importance and ensure preventive and corrective action is taken); an informed consent checklist (to verify that the procedures for informed consent meets all requirements); an informed consent verification log (to monitor that informed consent is correctly obtained from each participant); survey monitoring checklists and reporting templates (33). These tools, or selected elements thereof, may be adapted to complement the more specific tools provided in the annexes (see Annexes 2, 3, 10-14).

The survey coordination team should have control of all essential documents generated before, during and at the end of the survey (33). Essential documents are those which permit evaluation of the conduct of a survey and the quality of the data generated (33). This includes the survey documents outlined above as well as any additional documents that would allow for reconstruction of the survey, such as survey correspondence (including letters, memos, emails, meeting minutes) and the final survey report. Prior to starting a survey, a checklist should be developed listing all essential documents (33), and a system for tracking amendments through version control should be established.

5.2 Survey governance

Parties contributing to the global management of the survey and its implementation are typically organized into three levels as follows:

- Scientific advisory committee: This is a technical oversight committee which provides supervision of the overall conduct of the survey. The committee reviews and approves the protocol, the study data analysis plan, the publication of the study results and other survey documents. In the context of drug resistance surveys, technical oversight is often provided by topic experts (such as WHO,

¹ If additional testing will be performed outside the country, a Material Transfer Agreement (MTA) should be put in place to define the rights, responsibilities, and obligations for the use of the material (samples) coming from the provider (for instance, the Central Reference Laboratory) by the recipient (for instance SRL) for research purposes. The MTA should regulate aspects such as the permitted use, ownership, publication, intellectual property, and liability of the shared materials..

SRLs or non-governmental organizations) rather than through a formal scientific advisory committee.

- Survey coordination team: This team is responsible for the operational oversight and the day-to-day management of the survey. Depending on the country, the survey coordination team - chosen to manage the survey and provide direction - may also be referred to as the steering committee. Further information is provided in section 5.3 (Forming a national survey coordination team).
- Survey field team: This team is composed of central, regional/district and peripheral personnel tasked with implementing the survey at the field level, such as staff of health facilities and laboratories. The field team reports progress and challenges to the coordination team and ensures the scientific and ethical integrity of the survey.

5.3 Forming a national survey coordination team

A survey involves three major operational areas:

- programme management (logistics, training, collection of clinical information, supervision and monitoring of survey);
- standardized laboratory techniques and quality assurance procedures; and
- data and statistics (sampling design, data management and analysis).

A national survey coordination team, including experts from each of the above areas, should be established. In general, the coordination team includes the manager of the national TB programme (or designated persons), the head of the Central Reference Laboratory (or designated persons), an epidemiologist and/or a statistician, a logistician, and representatives from an academic or research institute. This team is responsible for quality assurance throughout the survey, including ensuring the necessary preparation, overseeing training, supervision, and monitoring, communicating with technical partners, and verifying the appropriate and timely analysis of data and reporting of results. The coordination team will require strong official backing from the government authority responsible for health services. A clear outline of team members and specific roles and responsibilities should be developed as part of the communications plan and the delegation log (see section 5.1: Survey documents and other essential documents). The person supervising and coordinating the day-to-day activities of the survey (survey coordinator) should not have any concurrent responsibilities for activities outside of the survey.

5.4 Setting objectives

Identification of specific survey objectives is a critical component of the initial planning process, because these will guide the development of a survey capable of collecting meaningful information. Objectives must be developed in the context of the available resources, funding and laboratory capacity in the area under study. It is important to carefully define the population of interest, as special approaches may be needed to capture meaningful data relating to certain subgroups, such as children, prison inmates, mobile populations, or patients seeking care in the private health sector.

A secondary aim of a drug resistance survey should be development or strengthening of a quality-assured laboratory network. A survey should enhance the existing diagnostic capacity in the country and lay the foundation for the establishment of continuous surveillance systems (see section 1.1: Continuous surveillance systems based on routine DST).

Specific objectives of the survey may include:

- to determine the prevalence of resistance to rifampicin, isoniazid, and other anti-TB drugs used in the treatment of TB among new and previously treated bacteriologically confirmed cases of pulmonary TB;
- to determine the prevalence of resistance to fluoroquinolones, new drugs (for example bedaquiline and delamanid) and re-purposed drugs (for example clofazimine and linezolid) among pulmonary cases of RR-TB;
- to determine the prevalence of resistance to fluoroquinolones among Hr-TB pulmonary cases;
- to investigate associations between phenotypic resistance and genotype;
- to describe the genetic traits of circulating strains of *M. tuberculosis*, which may allow identification of transmission chains, clusters and outbreaks and mutations potentially missed by commercially available molecular assays;
- to evaluate associations between drug resistance and characteristics such as age, sex and HIV status as well as other sociodemographic and clinical factors (see section 2.2: Age groups, sex, HIV status and other patient sociodemographic and clinical factors for limitations relating to survey design); or
- to monitor trends in drug resistance over time.

A survey typically involves enrolling a sample of bacteriologically confirmed pulmonary TB patients in order to characterize the drug resistance profile of a population. A bacteriologically confirmed pulmonary TB patient is defined as a person from whom sputum is positive for *M. tuberculosis* complex by smear microscopy, culture or a WHO-approved rapid diagnostic, such as Xpert MTB/RIF. Most commonly, an individual is enrolled in a survey at the place of initial diagnosis, regardless of whether treatment initiation occurs at the same site or not. However, depending on the organization of the health care system and laboratory network, some settings may choose to enrol patients at the facility where treatment is initiated, regardless of the place of initial diagnosis.

5.5 Defining the laboratory algorithm

With the ongoing development and uptake of new molecular technologies and next-generation sequencing, an expanding range of laboratory tests is available. In light of available resources, funding, and laboratory capacity, a survey diagnostic algorithm should be defined in consultation with the partner SRL which allows the survey objectives to be achieved. The algorithm can include a combination of sputum smear microscopy, culture-based methods, molecular technologies and sequencing, and should be adhered to throughout the survey. A flowchart is helpful in defining

the order in which the different tests should be performed, on which samples, and at which level (health facility, regional centres, Central Reference Laboratory or SRL). Examples of algorithms used in surveys in different settings are given in the box below and in Annex 1.

The selection of drugs to be tested depends on the survey objectives, logistical considerations, and treatment regimens in use in a given setting. Ideally, DST should be conducted for all anti-TB medicines used in the setting using a combination of phenotypic, molecular and/or sequencing-based methods. All bacteriologically confirmed pulmonary TB cases should undergo DST for rifampicin, isoniazid and other first-line drugs. All rifampicin-resistant and/or isoniazid-resistant pulmonary TB patients should be tested for susceptibility to at least fluoroquinolones. Additionally, RR-TB cases should be tested for resistance to other Group A drugs recommended by WHO for the treatment of RR-TB (such as bedaquiline and linezolid) (11) and for other selected drugs from Group B and C.

TAILORING SURVEY LABORATORY ALGORITHMS TO DIFFERENT SETTINGS (SEE ANNEX 1)

Democratic Republic of the Congo (DR Congo): Microscopy, Xpert MTB/RIF and targeted NGS

The first national survey conducted in DR Congo (2016-2017) tested all sputum smear-positive pulmonary TB patients by Xpert MTB/RIF. For those that were positive to *M. tuberculosis* complex, targeted NGS was performed by the SRL directly on sputum preserved in ethanol. This was the first national survey to rely entirely on NGS from sputum, providing resistance profiles for a range of drugs while bypassing the need for culture. It serves as a proof-of-concept for other settings that do not yet have rapid specimen transport networks or capacity to conduct culture.

Eswatini: Xpert MTB/RIF, culture and WGS

The third national drug resistance survey undertaken in Eswatini (2017–2018) utilised a comprehensive laboratory algorithm that incorporated rapid molecular tools, culture and sequencing. As per national guidelines for the diagnosis of TB, all presumptive pulmonary TB patients were tested using the Xpert MTB/RIF assay. Samples that were positive for *M. tuberculosis* complex were cultured in liquid media (MGIT), followed by WGS performed by the SRL. A key finding of this survey was that Xpert MTB/RIF missed half of the RR-TB cases in the country, due to a circulating clone with a specific mutation in the *rpoB* gene. The national TB programme subsequently modified their national diagnostic algorithm accordingly, to improve detection and ensure access to appropriate treatment and care. The results of this survey highlight the benefits offered by NGS for the surveillance of drug-resistant TB, including valuable insights into phylogenetics, strain evolution and transmission.

Myanmar: Microscopy, Xpert MTB/RIF, culture, line probe assay and WGS

In the fourth national drug resistance survey in Myanmar (underway since 2020), presumptive pulmonary TB patients were tested by Xpert MTB/RIF, either initially or following prior examination by sputum smear microscopy (regardless of the microscopy result). The hybrid entry point to the survey (microscopy and/or Xpert MTB/RIF, without the requirement for both) allowed a flexible approach to patient enrolment in view of the diagnostic capacity of the local health facility. Samples that were sputum smear-positive and/or positive to *M. tuberculosis* complex by Xpert MTB/RIF were cultured in MGIT and solid media (Löwenstein-Jensen) in parallel at two national reference laboratories. First- and second-line LPA on culture isolates is underway in these laboratories, and WGS will be performed at an international laboratory.

Togo: Microscopy, Xpert MTB/RIF, culture and line probe assay

The first national survey conducted in Togo (2017) tested all sputum smear-positive pulmonary TB patients by Xpert MTB/RIF. Samples that were resistant to rifampicin were cultured on solid media (Löwenstein-Jensen) followed by first-line phenotypic DST. Culture isolates that were resistant to rifampicin by phenotypic DST, or that were obtained from sputum samples that were resistant by Xpert MTB/RIF, were sent to the SRL for first-line and second-line DST by phenotypic methods (MGIT) as well as line probe assay. By restricting the full panel of DST to only those samples with rifampicin resistance, logistic challenges for sample transport and workload were reduced. However, only patterns of resistance linked to rifampicin resistance could be investigated.

5.6 Development of a protocol and time schedule

A comprehensive list of the sections and sub-sections that should be included in protocol development is given in Annex 2.

Once participating health facilities have been identified by the chosen sampling method (see section 5.8: Sampling of cases), a timeline can be established (Annex 2). The patient enrolment period usually takes place over 6–12 months, with a total survey time from protocol development to dissemination of results of approximately 18–24 months. All laboratory methods and the system of quality control and quality assurance should be discussed and agreed upon with the partner SRL. Furthermore, the protocol should describe ethical issues, and the established timeline should take into consideration the time required for the protocol to receive necessary approval from ethical review panels. An experienced epidemiologist and/or statistician must contribute to the development of the protocol to ensure the survey design will meet key objectives.

WHO, SRLs and other technical partners (and/or the scientific advisory committee where applicable) can assist in the development of a survey protocol, and should be asked to review a survey protocol prior to initiation of a survey. This will ensure that all requirements have been considered and described comprehensively; that quality control measures are in place; and that the data collected will be representative of the geographically-defined population under study. Once finalized, such a protocol should be distributed to all members of the survey coordination and field teams and health staff participating in the survey.

5.7 Minimum required facilities for a survey area

The country, province or city selected to be the target geographical area of a survey should have at least one quality-assured central laboratory for the selected testing methods (a Central Reference Laboratory, which is usually the National Reference Laboratory) linked to all intermediate TB laboratories, and most health facilities should have TB diagnostic capacity. If a quality-assured central laboratory does not yet exist, the shipping of sputum samples to an external laboratory may be considered.

Diagnostic and/or treatment centres

Patients should be sampled from sites where individuals with presumptive pulmonary TB are screened for TB. Most of these will be non-specialized health facilities or outpatient departments of hospitals operated by the government. A quality-assured laboratory and referral system network for accurate bacteriological confirmation of TB is a prerequisite for the implementation of a drug resistance survey.

The roles of all relevant healthcare providers (informal, public, voluntary, private) in the diagnosis and treatment of TB should be carefully considered. Inclusion of care providers functioning outside the national programme may require particular attention to assuring quality standards in diagnostics, sampling, and data recording and reporting. Countries with a sizable private sector should seek to include private health facilities in the survey, in order to obtain results representative of the entire population of bacteriologically confirmed pulmonary TB patients. Public-private mix initiatives can serve as platforms to gradually involve the private-sector laboratories in drug resistance surveillance activities. The preparedness of health facilities to participate in the survey should be assessed prior to implementation of survey activities, and any shortfalls addressed (see Annex 12).

Central Reference Laboratory

The Central Reference Laboratory undertakes the identification of *M. tuberculosis* and also performs DST, by either molecular or culture-based methods. There may also be other intermediate laboratories in the network capable of performing reliable DST by molecular or culture-based methods. One of the main tasks of the Central Reference Laboratory is to ensure the quality of smear microscopy, molecular testing, and culture and DST performed by regional or peripheral units by establishing a regular “onsite” supervision programme for those units, and by providing training in, and quality assurance systems for, the laboratory procedures. An external quality assessment programme with a partner SRL will validate the results of susceptibility tests done by the Central Reference Laboratory and any other relevant laboratories.

Basic laboratory equipment and materials must be available and functional in the Central Reference Laboratory before the implementation of a survey. Drug resistance surveys should only be undertaken when the laboratories are deemed to have an appropriate level of biosafety (19,20) and are equipped with trained staff working with clear standard operating procedures and producing quality-assured data. It is important to note that drug resistance surveys will heavily increase the workload of the Central Reference Laboratory, and therefore sufficient capacity must be assured

before the survey begins. An assessment of the preparedness of the laboratory network (Central Reference Laboratory and/or other relevant laboratories) must be conducted prior to the start of the survey (see Annex 11).

5.8 Sampling of cases

Statistical methodology is a fundamental aspect of the design of surveys. Accordingly, an experienced epidemiologist or statistician should be involved from the early planning stages.

5.8.1 Defining the sampling frame

The sampling frame for a survey depends on the objectives of the survey and the testing methods to be used. To measure the prevalence of drug resistance among new cases, the sampling frame should include all new bacteriologically confirmed pulmonary TB patients in the country that are identified by the routine national diagnostic algorithm (which may include sputum smear microscopy, WHO-approved rapid diagnostics, or a combination of both).

Active case-finding initiatives should be reviewed during the planning stage of the survey. Patients identified through community-based active case-finding that is linked to a health facility participating in the survey should be included in the survey. However, inclusion of results from screening of specific groups that may be at higher risk of drug resistance than the target population of the survey may introduce bias and therefore incorporation of these cases should be carefully considered. Any patient identified through active case-finding should be identified as such in the survey database to allow comparisons with those who self-presented to health facilities with signs and symptoms suggestive of pulmonary TB.

SAMPLING OF PREVIOUSLY TREATED CASES

Continuous surveillance of drug resistance among previously treated patients should be established as a priority in all countries. Accurate evaluation of resistance in previously treated patients provides crucial information for programme management. In settings where routine DST of previously treated cases is not yet in place, ideally a separate sample size calculation should be devised for a survey of previously treated patients. However, in most settings, achieving this sample size may require inclusion of more health facilities in the survey or extending the period of enrolment, adding to the complexity and cost of the survey. Instead, during the survey enrolment period for new patients, it is recommended that all previously treated patients who present to study sites be enrolled. Due to the small number of cases, estimates in previously treated cases are likely to be less precise than estimates in new cases and differences between subcategories of retreatment cases may not be interpretable.

5.8.2 Sample size

The main outcome of interest in surveys is the prevalence of RR-TB among new bacteriologically confirmed pulmonary TB cases. Estimations of the prevalence of resistance for other tested drugs will therefore be less precise than those for rifampicin.

The calculation of an appropriate sample size should be based on the following (34):

- the total number of new bacteriologically confirmed pulmonary cases registered in the previous year in the country or in the geographical setting to be studied;
- the expected prevalence of new bacteriologically confirmed pulmonary cases with RR-TB, based on available data (in the absence of available data, an informed estimate must be made); and
- the desired precision of the estimate, to be expressed as a 95% confidence interval. The sampling uncertainty should be as low as possible, while ensuring that the corresponding calculated sample size is logistically feasible. For example, if the prevalence of new cases with RR-TB is expected to be 4%, an absolute precision of 0.5% (0.005) means that the estimate may err within 0.5% of the true prevalence, corresponding to a 95% confidence interval from 3.5-4.5%.

The following formula can be used to calculate the sample size under simple random sampling, with a finite population correction:

$$n = \frac{N * z^2 * p * (1 - p)}{d^2 * (N - 1) + z^2 * p * (1 - p)}$$

where:

N = total number of new bacteriologically confirmed pulmonary TB cases registered during one year in the country;

z = z-value (from the standard normal distribution) that corresponds to the desired confidence level (if confidence interval =95%, $z= 1.96$);

d = absolute precision (as a decimal, for instance 2% should be expressed as 0.02);

p = expected prevalence of RR-TB in the target population (as a decimal, e.g. 4% should be expressed as 0.04).

The relative precision can be calculated by $\frac{d}{p} * 100$, and should ideally not be greater than 25% of p , where feasible, and never greater than 50% of p . For example, if the absolute precision is 0.005 and the expected prevalence of new cases with RR-TB is 0.04, the relative precision is $0.005 / 0.04 * 100 = 12.5\%$.

If the cluster sampling method is adopted (see section 5.8.3: Sampling strategies), the correlation between individuals within a cluster needs to be considered. In general, the design effect due to clustering in drug resistance surveys ranges from 1.5 to 3. Unless the design effect can be estimated from previous surveys, a design effect of 2 may be assumed, in which case the calculated sample size obtained from the equation above must be multiplied by 2.

While multiple imputation is recommended to reduce bias due to missing data (see section 7.2.1: Imputation of missing values), this will reduce the precision of the estimate of the prevalence of patients with RR-TB. It is therefore recommended to increase the calculated sample size to account for potential losses. Losses include bacteriologically confirmed pulmonary TB patients who do not provide consent to be enrolled in the survey, do not produce an adequate sample for the survey, or for whom a DST fails. For surveys relying on phenotypic DST, a loss of 10-20% of patients should be incorporated into the sample size calculation; for surveys relying on molecular DST for at least rifampicin, 5-10% may be appropriate.

As mentioned in section 5.8.1: Defining the sampling frame, the sample size calculation for previously treated cases is unlikely to be achievable due to lower numbers of notified previously treated cases. Instead, previously treated cases should be consecutively enrolled until the target sample size for new patients is reached.

5.8.3 Sampling strategies

For each repeat survey in the same country, sampling needs to be redone every time, using the most recent data available from the complete and updated list of health facilities.

A representative sample of all TB patients in the country is necessary to ensure that survey results are applicable at the national level. Cluster sampling methods are appropriate when it is logistically difficult to cover all health facilities where TB patients are diagnosed across the country. Patients from the same health facility form one cluster. The optimal number m of clusters depends on the variability of the prevalence of drug resistance between and within clusters, and the cost of including an additional cluster compared with the cost of increasing the size of an existing cluster.

Although more practical, a cluster sampling approach incurs sampling design effects that increase the variance of estimators when compared to simple random sampling. This is because patients in the same facility tend to be more alike than patients in different facilities. Therefore, the total amount of information contributed by n patients in m clusters is less than the amount of information from the same number of patients selected through simple random sampling. The lower the value of m , the greater the design effect. To retain the desired precision of estimators, the sample size needs to be inflated to the anticipated order of the design effect.

Health facilities may be randomly selected from a national list of facilities. Alternatively, sampling may be carried out in stages. For instance, starting with the selection of districts, followed by facilities within selected districts, may be more practical if there is no up-to-date national list of facilities.

Cluster sampling will be more efficient (leading to more precise estimators at a given size) if it is self-weighted; that is, if all patients have the same probability of being included in the sample. This can generally be achieved in one of two ways. The first method involves sampling health facilities with a probability proportional to size (PPS) approach (size is defined by the number of bacteriologically confirmed pulmonary TB patients in the previous year) followed by recruitment of a fixed number of patients in each selected facility (fixed cluster size, n/m). The second approach involves sampling

health facilities using equal probability, followed by recruitment of a variable number of patients per facility in proportion to their size (variable cluster size), usually achieved by enrolling all consecutive patients over a fixed period of time of the same duration in all selected facilities.

The most useful sampling strategies are described in more detail below. Examples of codes to be used for sampling are available for download from the website of the WHO Global TB Programme at <https://www.who.int/health-topics/tuberculosis> and <https://github.com/GTB-DRS>.

Exhaustive sampling of all health facilities

This sampling method is most suitable for small countries with relatively small numbers of health facilities diagnosing TB patients and comprehensive systems to transport samples to the Central Reference Laboratory. All eligible patients presenting to each health facility in the country are enrolled within a defined time period. Health facilities diagnosing very few cases per year (for example, less than 5-10 cases annually) may be excluded on the basis of logistic and feasibility considerations, provided that their exclusion does not result in sampling bias such as exclusion of a large proportion of the eligible patient population or disproportionate geographical or private-public sector distribution of eligible health facilities.

The self-weighted character of this design is ensured by including all facilities and using the same enrolment period within each of them. This approach will not incur the sampling design effects that occur in clustered surveys, and the sample size does not need to be inflated. The intake period is calculated by dividing the sample size n by the total number of bacteriologically confirmed patients per year in the country. For example, if approximately 5,000 eligible patients are diagnosed per year, and a required sample size of $n=700$ patients has been calculated, the enrolment period would be $700/5,000 = 0.14$ years or nearly two months. In this case, all consecutive eligible patients presenting to any health facility over a two-month period should be included until the national target of 700 is achieved, which provides a 14% sampling fraction of newly diagnosed sputum smear-positive pulmonary TB patients. Since the sampling fraction is relatively high, applying a finite population correction will reduce the standard error and improve the precision of estimators.

Cluster sampling

Fixed cluster size

This technique involves sampling from a list of health facilities where the probability of selecting a facility is proportional to its annual caseload (PPS approach). Sampling without replacement of health facilities will lead to more precise estimators than sampling with replacement.

This sampling strategy is suitable when the time needed to recruit n/m patients will not exceed 6–12 months in any of the selected facilities. Very small facilities with only a low number of notified cases should be regrouped with other facilities before sampling such that all facilities have a caseload of at least n/m over the desired maximum period of enrolment. Sampling with constant cluster size is easily implemented using a computer language such as R.

Variable cluster size

This strategy is more suitable if there are many facilities with a small number of patients being diagnosed during the course of one year. The number of patients to be enrolled in each facility is proportional to its caseload to ensure that the final sample is self-weighted. This is achieved by ensuring that all facilities enrol patients for the same period of time. As for the above sampling approach with fixed cluster size, sampling with variable cluster is easily implemented using a computer language such as R.

CLUSTER SAMPLING: ADAPTATIONS FOR HEALTH FACILITIES WITH LOW CASELOADS

Drug resistance surveys are discrete cross-sectional studies where enrolment of patients is completed within a relatively short timeframe. If small health facilities with only a low number of diagnosed new cases (for example, less than ten per year) are selected as clusters, the required sample size will not be achieved. If small facilities are very rare, these facilities can be excluded from the sampling frame prior to cluster selection provided that this does not result in exclusion of more than 10% of eligible patients and that the final sampling frame remains geographically representative.

If small facilities are common, their removal will introduce a selection bias. In this case, different strategies could be considered:

1. Neighbouring small facilities may be grouped together and considered as one unit in the sampling frame prior to cluster selection by PPS.
2. Geographical units (for example districts) may be sampled using PPS, and all health facilities in each unit must then enrol consecutive patients until the target sample size for the cluster (geographical unit) is achieved. This will require good communication and coordination between facilities belonging to the same cluster.
3. A variable cluster size approach could be considered, as it may be less logistically challenging and resource-demanding than fixed cluster size.

Multi-stage sampling

To minimize the logistical challenges that may arise in large countries with geographically dispersed populations, a multi-stage cluster sampling approach may be appropriate. The population is first divided into large groups, and a sample of these groups is taken, followed by progressive sampling of smaller units from these selected groups. For example, provinces or districts may first be selected as the primary sampling unit, followed by selection of health facilities within these as secondary sampling units, and then enrolment of consecutive patients in the selected facilities. The optimal multi-stage design depends on the context and should be considered on a case-by-case basis. Multi-stage sampling may involve a combination of different approaches such as sampling with and/or without replacement of sampling units, PPS and/or constant probability sampling, and either fixed or variable cluster size.

Stratification

In some settings, it may be appropriate to divide health facilities into two or more strata according to their annual caseload (for example small facilities, medium facilities, and large facilities). The national sample size is proportionally distributed to each stratum according to its share of the annual caseload. Within each stratum, sampling of health facilities is then conducted independently using either simple random sampling or PPS. Combinations of different sampling strategies can be used as appropriate. For example, in a stratum of large health facilities, PPS with replacement may be a suitable approach for selecting clusters of fixed size. In a stratum of small health facilities, simple random sampling without replacement or exhaustive sampling of all health facilities may be suitable, with each facility enrolling patients for the same period of time until the sample size for the stratum is achieved (variable cluster size). Selection of a greater number of facilities per stratum will result in faster completion of enrolment but may be more logistically challenging.

If there is evidence or suspicion of large differences in the prevalence of drug resistance between specific geographical areas (north versus south, or capital city versus the rest of the country) or populations (prisoners versus general population), stratification by these factors will improve the precision of estimators.

It might be necessary to include certain health facilities or geographic regions in the survey because of the critical role they will play in the diagnostic algorithm and workflow. For example, facilities equipped with GeneXpert instruments may be designed as regional hubs for testing, thus decentralizing work from the Central Reference Laboratory. In this situation, the sample could be stratified into two groups – (i) GeneXpert sites; (ii) other health facilities.

Example

A survey is being designed based on 40 clusters of variable size, to be selected from a total of 200 health facilities in a country, to achieve a target sample size of 2000 new bacteriologically confirmed pulmonary TB patients. Ten of these facilities are equipped with GeneXpert and, for logistic reasons, these should be included among the selected 40 clusters. As these 10 facilities accounted for 20% of new bacteriologically confirmed pulmonary TB cases notified in the previous year, they should collectively enrol $0.20 \times 2000 = 400$ new cases in the survey. The remaining 30 clusters should be chosen from the other 190 health facilities and should collectively enrol $0.8 \times 2000 = 1600$ new cases.

5.8.4 Repeat surveys

In general, the objective of a repeat survey is simply to obtain an updated estimate of the prevalence of resistance among bacteriologically confirmed pulmonary TB cases for planning purposes. If the objective were rather to detect a significant change in the prevalence between surveys, the required sample size would usually be much higher - the smaller the difference to detect, the larger the required sample size. This approach is logistically challenging and not recommended.

With the expanding range of laboratory diagnostic tests available and their incorporation into revised national TB diagnostic algorithms, the laboratory methods used in repeat surveys are likely to differ from those of a previous survey. To obtain an updated estimate, the integration of molecular technologies will reduce bias by: (i) increasing sensitivity of the diagnosis of TB, thus more accurately capturing the TB patient population; (ii) reducing losses arising from negative or contaminated cultures.

5.9 Budgeting

The required budget must be carefully calculated and all necessary funds for the entire survey period (planning, implementation, analysis and dissemination) must be available before the survey is started to avoid any interruption during survey implementation. See Annex 6 for a template of a survey budget.

National TB programmes should consider surveys not only as a means of estimating the magnitude of drug resistance, but also as an important tool for monitoring programme efficiency, and as a means of strengthening the capacity of the Central Reference Laboratory, laboratory network, sample referral systems, and data management. Therefore, the allocation of funds towards surveys should be an integral part of a programme's budget.

The current average cost of nationwide surveys is around \$US 200,000–400 000, based on an average sample size of approximately 1,000–2,000 patients. However, this will vary according to setting and laboratory testing algorithm. Inclusion of NGS may increase the budget beyond this range, but costs may decrease in the future.

All budgets should include the costs for technical assistance from an SRL, including costs of retesting isolates and all other laboratory work, and costs of shipments to and from SRLs for quality assessment of specimens/isolates. There may also be important costs associated with the staffing required to process specimens and/or laboratory running costs.

Costs for the overall coordination of the survey should also be included. This may include staff salaries, training, coordination meetings, monitoring and supervisory visits to health facilities, and communication between the peripheral centres and the Central Reference Laboratory and data management unit. Costs of external technical assistance should also be included, as appropriate.

5.10 Training

To help deliver training effectively, a plan outlining induction and ongoing training needs (topics to be covered in training, requirements for refresher training, etc.) may be developed, with details of how, when and by whom the training will be carried out. Training packages for survey processes and procedures should be developed with due consideration of relevant aspects related to ethics and good clinical practice and good data management practices (33). Training may be delegated to qualified trainers following training of trainers. To monitor induction and continued training of staff, all training activities should be documented in training logs. Where possible,

a competency assessment should be used to evaluate the effectiveness of the training and ensure learning objectives were met. This could take the form of a simple quiz or an actual demonstration and evaluation of skills at the bench.

Training should focus on the following essential parts of the survey:

- enrolment of eligible patients in the survey, and obtaining reliable and comparable data on patient history of previous treatment;
- informed consent or informed assent where appropriate, and respect for confidentiality;
- specimen collection and transportation;
- use of data collection forms;
- laboratory techniques;
- communicating results back to the health facility (and, in turn, to the patient);
- data entry, validation and analysis;
- survey supervision, monitoring and communication strategy; and
- relevant SOPs specific to the individual's role in conducting the survey.

Involvement of the national TB programme in all training activities will improve engagement of relevant healthcare workers. The survey coordination team, as well as central, regional/district and peripheral field teams, should receive training on conducting supervision and ensuring communication with facilities. The medical officers/nurses in charge of patient intake and interviews should be identified and properly instructed in each health facility involved in the survey. A meeting can be an efficient way to inform, train and motivate the officers involved. Remote training materials are also valuable for new staff joining health facilities during the course of survey implementation, after initial training has been conducted. This could be in the format of slide presentations accompanied by audio that can be viewed offline without an internet connection.

Training at peripheral laboratories should be considered for registration of samples, sputum smear microscopy, molecular testing methods, decontamination of sputum samples for culture, storage and transport, and recording of results. Monitoring of quality indicators will help inform decision-makers on training needs and required action at any point during the survey.

5.11 Laboratory network preparedness

The Central Reference Laboratory should establish a comprehensive quality assurance programme to ensure the quality of testing of the samples. With support from regional teams where appropriate, the Central Reference Laboratory must undertake visits to peripheral laboratories in the network to assess preparedness prior to the survey and to monitor survey implementation, and provide training if internal quality control procedures are insufficient. The collection of sputum samples (including sputum quantity and quality), sputum smear examination, peripheral molecular testing, and transport of sputum samples and forms must all be reviewed and monitored.

Undertaking a survey may place considerable pressure upon the peripheral laboratories and the Central Reference Laboratory. Laboratory logistics, facilities, and resources necessary for a survey must be considered in advance, so that the laboratory network is not overwhelmed by the extra workload and routine activities remain unaffected.

Before the survey begins, the Central Reference Laboratory should undertake, in collaboration with a partner SRL, a preparedness assessment (Annex 11) to evaluate all of the key aspects of the quality assurance programme and ensure that certain measures are in place. The following must be assured:

- adequate biosafety measures are in place according to the testing algorithm;
- all equipment required to test and store the survey samples is functional and properly maintained and calibrated;
- adequate laboratory supplies are available;
- SOPs covering all the steps and procedures of the diagnostic algorithm are in place;
- the staff involved in the survey are deemed competent for the role;
- the sample referral system is functional and efficient; and
- adequate internal quality controls are in place.

In addition, the assessors should review and analyse the Central Reference Laboratory's routine quality indicators for all the testing methodologies that will be used in the survey. They should also review and analyse results of external quality assurance testing, ensuring that effective corrective measures have in been put in place whenever needed.

Proficiency testing coordinated by the SRL for phenotypic and genotypic DST must be completed with good results before the survey begins. The relationship between the Central Reference Laboratory and the partner SRL should be continuous and responsive to any substandard performance that may be detected during the course of a survey. An SRL may be required to recheck more or fewer samples than initially planned, depending on the Central Reference Laboratory's performance.

5.12 Pilot study

Depending on local conditions, it can be helpful to organize a time-limited (for example one month) pilot study in several of the chosen sites in order to test the entire process of patient identification and classification, sputum collection, processing and shipment, laboratory testing, documentation and coordination, and the quality of training. The pilot study can serve to identify and solve unexpected problems before the survey is launched in all sites. If no significant problems are encountered during the pilot period, the data collected can be included as part of the survey and contribute to the necessary sample size.

6 Survey implementation

The logistic aspects of the survey will depend on patient inclusion and exclusion criteria and the diagnostic testing algorithm being used.

6.1 Inclusion and exclusion criteria

Inclusion and exclusion criteria are defined according to the population of interest described in the survey objectives. Most commonly, a patient is eligible to be included in the survey if diagnosed and registered as a new or previously treated bacteriologically confirmed case of pulmonary TB (see section 2.1: Patient treatment history classifications) at a health facility selected to participate in the survey, regardless of whether they will receive treatment at that facility. This may also include patients detected through active case-finding initiatives (see section 5.8.1: Defining the sampling frame). Patients who have started their current course of TB treatment more than one week earlier (meaning they are more than seven days into their current treatment course) should be excluded from the survey.

RR-TB in children can be an indicator of recent transmission of drug-resistant strains from contacts present in their environment (35). Children under 15 years old who meet the admission criteria should therefore be included in surveys subject to the participant's assent and in accordance with the national laws of the country. The use of Xpert MTB/RIF and other rapid molecular tests may reduce some of the challenges of diagnosis in children (18, 19).

Extrapulmonary TB and clinically diagnosed pulmonary TB cases (those who do not have bacteriological confirmation but are given a full course of treatment based on clinical signs and symptoms) are excluded from surveys due to difficulties in diagnosis and resource limitations.

6.2 Patient enrolment

Each patient who meets the inclusion criteria and provides informed consent or assent to participate in the survey should be assigned a unique survey identification number that will be used on all patient forms, including the case report form, laboratory forms (for example sputum shipment and results forms) and the sample container. For example, the survey identification number could be based on a code representing the health facility where the patient was enrolled, followed by consecutive numbering of each patient enrolled at that centre. Each survey identification number is linked to only one person, and each participant is identified by only one number. Ensuring that basic patient information such as age and sex are recorded on each form can be important for identification, particularly in the event of that the same survey identification number is erroneously attributed to two different patients.

The survey identification number allows data collected on different forms to be linked in the survey database. It also enables the identification of the patient at the health facility in the event of a drug-resistant strain or when additional information is required. An enrolment log (33) linking survey identification numbers and patient

details in routine registers should be kept at the enrolling health facility for clinical management. If specific codes are already in use for the identification of administrative areas or health facilities, these codes can be included as a component of the unique survey identification number.

All patients meeting the inclusion criteria should be offered the opportunity to participate in the survey, and enrolled if informed consent or assent is given. The process of enrolment involves the following: informing the patient of the survey, consenting or assenting the patient, offering an HIV test in accordance with existing national policies, interviewing the patient and reviewing the medical records to complete the patient case report form (see Annex 7), assigning the unique survey identification number, recording the patient in the appropriate register for tracking purposes (for example enrolment log), and collecting and submitting sputum samples for use in the survey prior to the commencement of treatment. The number of sputum samples required may vary according to the diagnostic methods being used (see section 6.3: Sample collection, storage and transport). As a measure of quality control, the number of eligible patients (computed from routine registers held in the health facility) and the number of patients actually enrolled in each survey site should be compared regularly during the enrolment period (see Annexes 12 and 13). This can help to identify reasons why some patients may not have been enrolled in the survey, and reduce the likelihood that eligible patients are missed.

Surveys may be implemented so that enrolment begins simultaneously in all health facilities, or staggered with facilities beginning at different times. In a survey utilising exhaustive sampling of all health facilities, enrolment could be undertaken either during the same month or on rotation – for example, facilities in zone one during the first two months, facilities in zone two during the following two months, and so on. In this way, the number of sputum samples to be tested by the Central Reference Laboratory would remain stable throughout the year, to avoid overloading staff and equipment. Countries that have recently completed surveys using exhaustive sampling of all health facilities in a staggered approach include Malawi and Mali.

In clustered survey designs, a staggered approach may be used to progressively increase the numbers of patients enrolled in the survey each week, to allow the Central Reference Laboratory to gradually transition into survey procedures. As the length of enrolment period can vary substantially between clusters with fixed sample sizes, a staggered approach can ensure that a relatively similar number of patients are enrolled each month, maintaining a stable and feasible workload. This approach was followed in recent surveys conducted in Myanmar and the Philippines. The impact of any seasonal variations in caseload, however, must be carefully considered before adopting a staggered approach and the total time to complete enrolment should not exceed one year.

6.2.1 Case report form

The case report form is a printed or electronic document designed to capture all important information related to the participant. In the context of a drug resistance survey, the main objective is to correctly identify any previous treatment of the patient for TB.

The case report form (see Annex 7) consists of four categories of information:

- identification of the patient;
- sociodemographic information, including age and sex;
- other relevant clinical information such as HIV status; and
- history of previous treatment for TB as determined by interview and review of medical records, including any treatment regimen received and place of treatment (for example public/private).

This form collects a minimal set of information necessary for programme monitoring of treatment history classification, and for the possible analysis of risk factors for drug resistance. This information must be collected in every survey. Countries may decide to collect additional information as described in section 2.2: Age groups, sex, HIV status and other patient sociodemographic and clinical factors. In principle, only information that can assist analysis and is obtainable, reliable and useful from a programmatic perspective should be included; all included information must be well-described in the survey protocol. There must be a known denominator for each variable collected. For example, if pulmonary TB patients are to be stratified by country of origin, all patients must be asked to provide such information.

If data collection is paper-based rather than electronic, a copy of the completed case report form should be sent to the coordination team staff responsible for the management of survey data, with the original kept at the health facility and stored in line with confidentiality principles agreed with the local ethics committee. Booklets containing each form in duplicate separated by carbon paper can be a simple and fast way to make copies.

In the context of drug resistance surveys, the case report form is usually accessed only by survey staff that are also part of the programmatic and clinical management of TB (for example healthcare workers, staff of the national TB programme, laboratory staff). Depending on the country, it may be appropriate for the case report form to contain patient identifying information to ensure traceability of clinical records. Identifiable data must never be shared outside the programmatic and clinical teams and must be securely stored. Its inclusion in the case report form should be clearly justified as essential, and is subject to the approval from the relevant ethics committee(s).

QUALITY CONTROL OF CLASSIFICATION OF HISTORY OF PREVIOUS TREATMENT FOR TB

Classification of patients as being either new or previously treated is critical and has important implications for data analysis and interpretation. Special efforts are therefore needed during the survey to ensure the reliability of clinical data, and allow any updated information to be captured in the survey database.

Several questions should be included on the case report forms to help elicit an accurate treatment history through patient interviews and a review of medical records. The collected

forms should be checked carefully for deficiencies, and the reliability of the recorded information should be assessed regularly. Re-interviewing patients is one important method to verify treatment history. All patients with rifampicin resistance should be re-interviewed, particularly new patients. Measures should be taken to provide a comfortable environment for the interview and to eliminate any barriers or stigma that may prevent a patient from disclosing a truthful treatment history. It is possible that when patients begin feeling better after starting treatment, they may be more willing to provide details of their treatment history.

It is important to note that the proportion of cases classified as being previously treated is often found to be higher in surveys than through routine programmatic data. This may be because the comprehensive patient treatment history recorded in surveys reduces patient misclassification.

6.3 Sample collection, storage and transport

6.3.1 Collection, storage and referral of sputum samples

The correct collection, storage, referral and processing of sputum samples by the laboratories participating in the survey is essential to ensure the quality of results. Health care workers must be well trained in providing patients with clear instructions in order to collect a good sputum sample. Aerosols containing *M. tuberculosis* may be formed when the patient coughs to produce a sputum specimen (36). Patients should therefore produce sputum (not saliva) either outside in the open air or in special sputum collection rooms with appropriate ventilation and/or other methods to kill bacilli such as ultraviolet irradiation (36), always away from other people. Sputum collection should not be performed in confined spaces such as a room in the laboratory, or in the toilets. Ideally, at least two sputum samples should be taken from each patient enrolled into the survey, with a preferred sample volume of 2-5mL, depending on the tests used. However, it may still be possible to obtain results from samples of smaller volume and these should not be discarded.

Sputum should always be treated with care. Suitable containers must be robust to avoid being crushed in transit, and must have a watertight, wide-mouthed, screw-top to prevent leakage and contamination. Containers should be packed in material that will absorb any leakage caused by accidents. Before transport, sputum samples should be kept in a cool place, preferably refrigerated at +4°C. Cool boxes and triple packaging (see Annex 9) should be used to transport samples from the health facility to the laboratory. Recommended turnaround times from sample collection to sample processing should not be exceeded. For unprocessed cooled samples, these are ≤ 3 days for liquid culture, ≤ 5 days for solid culture and ≤ 7 days for molecular-based assays.

Ideally, barcodes should be used to identify and trace samples and to link to paper forms and electronic data capture tools. If barcodes cannot be used, a patient's unique survey identification number should be written on each container (not on the lid), as well as on the sputum collection and laboratory request forms. It is recommended that each sample from the same patient is identified uniquely by combining the patient

identification number with a code to identify the specific sample (for example first sample, second sample, etc). The standard laboratory forms which accompany sputum specimens during shipment and to request laboratory analysis should be modified as necessary for the survey. To ensure traceability, the health facility should keep a register of the following: survey identification number; date of sample collection; date of sample shipment; date of laboratory testing (if available) and receipt of laboratory results; and date of transmitting results to patient. A system to track what samples are received, processed, stored, lost or unprocessed at the Central Reference Laboratory, and/or subsequently shipped to SRL is needed. The Central Reference Laboratory should also keep a record of when test results are available and reported back to health facilities for the clinical management of patients. To this end, the Central Reference Laboratory may record the reception of samples using routine procedures, and should also complete a register detailing the processing, storage, shipment and reporting of survey results.

More detailed information about the requirements for specimen collection, storage, packaging, transport and documentation can be found in the GLI's *Guide to TB specimen referral systems and integrated networks* (37).

TRANSPORT SOLUTIONS AND COMMERCIAL TRANSPORT PRODUCTS

Biosafety concerns, preservation of mycobacterial viability and inhibition of growth of contaminating flora are key factors to consider when planning the referral of sputum samples for culture. In the likelihood of an unreliable cold chain system or when delays (greater than 3-4 days) in storage and transportation are anticipated, cetylpyridinium chloride (CPC) can be used to store the sample at room temperature and prevent the growth of contaminating flora. Samples treated with CPC solution should never be refrigerated because of the likelihood of crystallization at low temperatures. Samples in CPC should be shipped to the testing laboratory preferably within 7 days. The addition of CPC must be indicated on the accompanying documents because CPC must be removed by centrifugation before sample processing for either culture or molecular testing. Sputum samples containing CPC can only be cultured on egg-based media, not liquid or agar media.

There is currently no strong evidence that commercial products improve performance of molecular tests (38). However, for sputum samples transported for rapid molecular testing only, options for maintaining the stability of nucleic acids, but not mycobacteria viability, could be considered. Sputum specimens can be inactivated, and DNA stabilized by adding ethanol (70% final concentration), as has been successfully used in some surveys (39). Inactivated samples can be stored and transported at room temperature or at 2–8°C with minimal biosafety restrictions (see Annex 9).

Mitigation measures should be put in place to minimize the risk of loss of specimens or failure to produce interpretable DST results at the Central Reference Laboratory or at the SRL level. Risks include an interrupted power supply, the sudden

down-time of essential equipment, stock-out of laboratory reagents, or damage/loss of the specimens during referral. An uninterrupted power supply should be assured.

It is recommended to systematically store surplus sputum samples and sediments to allow repeat or further testing if needed. If space allows, sputum sediments and/or sputum samples should ideally be stored in a deep freezer at -20°C , or at -80°C if the conditions for storage at this temperature can be ensured (for example stable power supply to ensure no damage to cooling engine), until the final culture and DST results are available (see Annex 8). This maintains *M. tuberculosis* viability, so that culture and DST can be repeated in case of contamination. It also allows the long-term storage of sputum samples or sediments for subsequent molecular testing, including NGS. In the presence of ethanol, sputum samples can be stored at room temperature, out of the light.

6.3.2 Storage and referral of culture isolates

Although mycobacteria remain viable on solid or liquid media at room temperature for weeks to months, they are most reliably recovered from actively growing or fresh cultures. Bacteria may be subcultured on fresh media before shipping, although it should be noted that repeated sub-culturing may change the proportion of resistant bacteria. Alternatively, culture isolates can be refrigerated at $2-8^{\circ}\text{C}$ or long-term stored in a deep freezer at a temperature of at least -20°C but ideally -80°C . Deep freezing of culture isolates must be performed in liquid broth (for example 7H9) in the presence of 20% glycerol (Annex 8).

Before storing or shipping any culture isolate for DST, the purity of the positive culture must first be confirmed and the presence of any nontuberculous mycobacteria or other bacteria excluded.

Culture isolates should be shipped at room temperature in unbreakable cryovial with external-thread screw-cap as primary watertight containers (preferred option for international shipping) and packaged according to national and international regulations. Petri dish cultures and large volumes of liquid cultures must not be shipped. If glass tubes (such as those from Löwenstein Jensen cultures) are shipped, they must be packaged to prevent breakage.

Culture isolates are classified as Category A infectious substances (UN2814) and should be packaged according to the relevant requirements (P620, International Air Transport Association (IATA)), particularly when transporting by air (see Annex 9). However, for ground transportation, according to the *European Agreement concerning the International Carriage of Dangerous Goods by Road (40)*, cultures may be classified as Category B infectious substances when the cultures are intended for diagnostic or clinical purposes.

More detailed information on the regulation for transport of infectious substances can be found in WHO's *Guidance on regulations for the transport of infectious substances 2019–2020 (41)*.

Culture isolates undergoing WGS can be inactivated before shipping to the referral laboratory. Inactivated samples may not require conformance with requirements for the transport of infectious material, resulting in significant reduction in the costs for

international shipping (see Annex 9). Different inactivation methods exist that do not interfere with downstream molecular testing (see Annex 9). Importantly, the Central Reference Laboratory is responsible for storing a backup aliquot of the viable isolate in a deep freezer at least until re-testing at the SRL has been completed or the isolate has been excluded from needing further testing.

6.4 Monitoring and evaluation

A schedule for conducting monitoring visits to all participating health facilities should be developed as part of the monitoring plan (see section 5.1: Survey documents and other essential documents) and budgeted for before the start of the survey. At a minimum, all facilities partaking in the survey should be visited by the central coordination team and/or regional field teams at least once during the intake period. A monitoring form can be developed in advance which can be helpful for assessing the adherence of the staff to the survey procedures during monitoring visits (see example in Annex 12). After the visits, a flexible risk-based monitoring approach should be used. For example, health facilities can be contacted by phone every 1–2 weeks to remotely identify those requiring further supervision through additional monitoring visits and more frequent phone calls. A checklist can also be developed to ensure that the remote monitoring is conducted in a systematic way (see example in Annex 13). Facilities which are performing well may continue to be monitored remotely for the duration of the intake period following the initial visit.

At regular intervals (for example monthly) during the intake period, all data produced by the health facilities and laboratories should be tabulated and reviewed. The frequency of the data review can be increased following the identification of any incidents, adopting a risk-based approach to monitoring. The coordination team's epidemiologist should make regular reports based on these tables to the survey coordination team. These reports should include selected survey quality and progress indicators related to enrolment, completeness of data, transport and logistics, laboratory results and other issues (see Annex 14 for a comprehensive example list).

If monitoring activities and/or data reviews identify significant problems, the survey coordination team should develop a detailed plan for addressing these. Missing information should be requested from the respective centres as soon as possible after specimen receipt. Members of the survey coordination and/or field teams (as per delegated roles) must visit health facilities with low patient enrolment, incomplete data collection forms or delays in sample shipment.

Halfway through the survey, the national survey coordination team should hold a midterm review meeting to discuss the quality of data collection, laboratory procedures, quality control results, and preliminary survey results, including interpretation. Additionally, an external monitoring review should be conducted by experts who are not members of the survey coordination team (for example topic experts from stakeholders providing technical assistance; see section 5.2: Survey governance) close to the start date of the intake period but ensuring that sufficient data has been collected to allow a meaningful review.

7 Data management, analysis and dissemination of survey results

7.1 Data management

Data management is aimed at producing high-quality data on individual characteristics and aggregated indicators. Managing survey data appropriately ensures that the data are complete, reliable, and processed correctly, and that data integrity is preserved. Data management includes all processes and procedures for collecting, handling, cleaning, validating, analysing and storing/archiving data throughout the study.

The survey data management systems should address:

- data acquisition;
- confidentiality of data;
- data management training;
- completion of case report forms and other survey-related documents, and procedures for correcting errors in these documents;
- coding/terminology for patient characteristics and medical history (data dictionaries);
- electronic coding of missing values;
- database design and testing;
- programming edit and range checks;
- data entry and verification (for example random checks for errors);
- quality assurance for data quality and validity;
- database validation;
- secure, efficient and accessible data storage (including systems for regular version-controlled back-up storage of electronic records);
- database closure;
- storage of paper and archiving of electronic records after the study ends; and
- policies for data ownership and sharing.

Detailed guidance on this topic is available from WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys* (33). A database manager should be appointed to take charge of the process, including the development of a centrally-managed database. A plan documenting appropriate data management systems, which incorporate the principles of good data management practices, should be developed in advance (see section 5.1: Survey documents and other essential documents). The survey coordination team must take responsibility for implementing such systems to ensure that the integrity of the survey data is preserved. The data management plan describes the procedures and processes for ensuring the data conform to ALCOAC principles (for example attributable, legible, contemporaneous, original, accurate and complete) and are verifiable with source documents (primary data) that

match the data protocols in the survey. This includes consideration of aspects related to: monitoring the survey; transferring, sorting, entering, validating, and cleaning the data; and making the data available for analysis.

All patients enrolled in the survey should be entered into the database, regardless of whether their laboratory results are available. This includes patients whose samples were lost or contaminated. The data held in the database must be sufficiently comprehensive to allow the processes and analyses specified in the survey protocol to be performed, such as reporting the proportion of patients without DST results, allow reporting of key survey quality and progress indicators for monitoring purposes and to inform decision making performing multiple imputation of missing data (see section 7.2.1: Imputation of missing values). All data recorded on forms and in the database should use the same survey identification number to uniquely identify each patient and allow the linkage of different forms. Barcode labels and handheld scanners are recommended, as these reduce transcription errors. Labels should be prepared in advance and attached to the necessary forms and tubes for each patient. Where systems are already in place for the automatic electronic capture of testing results, for example from Laboratory Information Management Systems in Central Reference Laboratories or connectivity solutions for GeneXpert instruments, results should be directly imported from these databases using the unique survey identification number.

A relational database will ensure referential integrity. In such a database, data from the different data collection forms (for example patient case report form, laboratory results form) can be stored in separate tables, while ensuring that the data are consistently linked between tables using the unique survey identification number of the patient. Automatic validation checks should be built into the database to immediately identify errors during data entry, for example placing a restriction on the values which are allowed to be entered into a given field. Additional routine checks that can be regularly run, such as identifying outlier values requiring further investigation and verification, should also be included. Microsoft Excel is not a suitable software for entering, storing or managing survey data and it does not provide audit trails.

Survey teams are encouraged to use database software that is familiar to them. A fully customisable electronic data-capture tool for anti-TB drug resistance surveys is available in District Health Information Software 2 (DHIS2) as part of the TB country package. DHIS2 (42) is an open source, web-based health management information system platform designed in line with WHO standards for service delivery and programme implementation. The survey tool includes dashboards for visualisations and summaries of data, including for recommended quality and progress indicators. The user does not require expertise in database configuration and design. All countries are encouraged to move towards establishing electronic systems for recording and reporting of routine TB surveillance data, and the use of this survey tool may provide a first step towards establishing this. For more information about data management, see the 2011 WHO publication *Tuberculosis prevalence surveys: a handbook (15)* (revised edition expected in 2021).

7.2 Data analysis

The first step in data analysis is the development of a flowchart showing the outcomes of all of the eligible patients enrolled in the study (see example in Annex 4). This allows the identification of the steps where eligible patients were lost from the survey, which risks introducing bias. The flowchart should be disaggregated by patient treatment history and should contain boxes for the following: number of patients enrolled; number of patients for whom samples were not available for further testing (for example lost samples); number of patients for whom further testing was performed but results were not available (for example contaminated samples); and the numbers of patients with final DST results.

The following analyses of drug resistance data should be conducted:

- *Analysis of patient intake.* It is important to make a table comparing the number of patients included from each site with the expected number based on the sampling method, disaggregated by treatment history. Tabulations of data by site allow an assessment of the extent of missing data.
- *Analysis of missing value patterns.* DST results may be missing for a variety of reasons, including lost samples, contaminated samples, negative results for *M. tuberculosis* by molecular methods or culture, or insufficient culture growth for susceptibility testing. The percentage of enrolled bacteriologically confirmed patients for whom data on drug resistance to rifampicin and/or isoniazid is missing should be summarized by age group, sex, treatment history and site.
- *Analysis of drug resistance patterns.* It is essential to develop tables describing the prevalence of resistance, and associated confidence intervals, to individual drugs and to different combinations of drugs for new and previously treated cases (the most important being resistance to rifampicin, resistance to fluoroquinolones among RR-TB cases, and resistance to fluoroquinolones among Hr-TB cases). Tables of aggregated numbers of cases are shown in Annex 5. However, an estimate of the proportion of cases with drug resistance that is based only on those patients with a test result may be biased, as it assumes that patients with results are a random subset of all patients enrolled in the survey, which may not be the case. Therefore, statistical methods such as multiple imputation may be needed to reduce the risk of bias (see section 7.2.1: Imputation of missing values).
- *Analysis of determinants of resistance.* Depending on sociodemographic and clinical data collected, further comparisons based on sex, age groups, HIV status, country of origin, etc. should be evaluated (see tables in Annex 5).

Specialized statistical software is needed to analyse drug resistance data from national surveys with cluster sampling. The reason for this requirement is to account for missing data and sampling design effects on the estimates and their standard errors.

Practical steps for analysing a sample survey dataset are available for download from the WHO Global TB Programme website at <https://www.who.int/health-topics/tuberculosis> and <https://github.com/GTB-DRS>.

7.2.1 Imputation of missing values

Multiple imputation of missing data may reduce bias compared to an analysis based on only those patients for whom a DST result is available. However, multiple imputation should never be considered a substitute for initial collection of high-quality data.

In surveys, data are likely to be “missing at random” (MAR). This means that the probability that an individual has missing data for the outcome variable is related to individual characteristics such as age or sex; however, within groups of individuals who have the same age, sex, or other characteristics, the probability of data being missing for the outcome variable is not associated with its value (RR-TB or rifampicin-susceptible TB). For MAR data, multiple imputation of missing values should be performed, and the results compared with a non-imputed analysis.

If data are “missing not at random”, the probability of an individual having missing outcome data is different for individuals who have drug-susceptible TB compared to those that have drug-resistant TB. In this case, multiple imputation should not be performed and a sensitivity analysis is required, as the survey results may be biased.

Multiple imputation involves using the patterns within the available data to assign outcomes for patients missing data. Analysts can then apply the statistical method they would have used if there were no missing values (for example logistic regression). In general, we can be confident of obtaining an unbiased estimate of the prevalence of drug resistance among bacteriologically confirmed pulmonary TB cases if certain conditions are met.

- (i) The percentage of patients with missing data is low.
- (ii) The data are MAR.
- (iii) Appropriate imputation models are used.
- (iv) The data from imputed datasets are combined in an appropriate way.

95% confidence intervals should be calculated to account for the uncertainty introduced by the imputation. In the context of surveys, imputation is usually only performed for the primary outcome of RR-TB, for which possible predictors are better understood. Imputation may also be conducted for other drugs, subject to the proportion and patterns of the missing data within the dataset as a whole, and the likelihood of building a biologically sound imputation model that is based on meaningful potential predictors.

7.2.2 Sampling design effects on standard errors

Apart from addressing potential biases created by missing data, the second major feature of a cluster sample survey to be addressed in the analysis is the lack of statistical independence of observations from the same cluster. This arises because individuals within clusters are likely to be more similar to each other than to individuals in other clusters. This intra-cluster correlation (equivalent to inter-cluster variation) must be accounted for by inflating standard errors (and therefore widening the confidence intervals) around the estimated proportion of rifampicin resistance.

7.2.3 Sampling weights

In surveys involving exhaustive sampling of all health facilities or variable cluster size designs where the duration of the intake period is identical for all health facilities, the number of patients enrolled should be proportional to the caseload in each health facility. If this is not the case, weights may be applied to adjust for differences between the expected and observed number of enrolled cases. The expected number can be derived from routine notification data from health facilities for the same period in the current or in the previous year.

In fixed cluster size designs, weights can also be applied if the target cluster size is not reached. For example, if the planned cluster size had been 30 new patients per cluster but there was wide variation in the numbers of patients enrolled in some clusters, the weight assigned to each new patient within a given cluster would be equal to 30 divided by the actual number of new patients enrolled (for example $30 / 20 = 1.5$). The denominator should include all eligible cases, regardless of whether DST was successful and results are available. The final analysis should be carried out both with and without these weights, and differences in model coefficients should be examined carefully. If there is concern that the number of patients enrolled in a cluster might be associated with the proportion with rifampicin resistance, it will be important to make a correction in the analysis for this potential bias.

7.3 Dissemination of survey findings, and policy and practice implications

The reliable and comprehensive results generated from a drug resistance survey give rise to opportunities to facilitate multisectoral engagement and policy discussion; promote capacity-building; inform strategic planning; and trigger appropriate interventions. To effectively translate the survey findings into concrete national action, stakeholder consultations must take place to review survey results for contextualized interpretation and action planning. The national TB programme and the principal investigator may begin by holding small technical consultations to review the findings in detail, discuss operational challenges, potential biases, survey limitations, and lessons learned and jointly interpret the results in the context of country's overall progress towards managing and eliminating TB. During these initial technical consultations, key interventions should be identified to strengthen surveillance systems, expand laboratory networks, improve coverage of DST, and optimise treatment and care of patients with drug-resistant TB.

INTERPRETATION OF SURVEY FINDINGS

Drug resistance by age group

Younger people are more likely than older people to have been recently infected. The proportion of new cases with drug resistance among younger age groups may therefore provide more reliable information about recent patterns of transmission of drug-resistant TB and the overall quality of a national TB programme. However, the numbers of patients with drug-resistant TB in different age categories is often too small to allow the detection of significant differences.

Drug resistance by history of anti-TB treatment

Previously treated cases are a heterogeneous group. Drug resistance among previously treated cases may be the result of primary transmission; this may have been missed at the time of initial TB diagnosis or acquired during treatment. Various factors promote acquired resistance among previously treated cases. These include unsupervised treatment, inappropriate drug regimens, availability of anti-TB drugs without physician prescription or oversight, poor quality of the drugs supplied, and inadequate methods for declaring patients as successfully cured. A subgroup analysis (see section 2.1: Patient treatment history classifications) may provide more targeted conclusions and recommendations, although this may not be possible in surveys with only small numbers of patients.

Trends in drug resistance over time

Surveys should ideally be conducted every five years and trends over time should always be interpreted within the context of the overall programme. This includes consideration of other indicators such as treatment outcomes, changes in overall incidence of TB disease, prevalence of HIV, changes in standardized or empirical drug regimens, size and engagement of the private sector, major socioeconomic events and drug shortages. This allows for more robust interpretation of drug resistance surveillance data in a given setting.

Patterns of drug resistance

Surveys provide comprehensive drug resistance profiles for different patient groups and, depending on the laboratory algorithm used, the underlying molecular mechanisms. This information should be used to identify any necessary modifications to the national diagnostic algorithm for TB, including the specific drugs to be tested, the type of tests to be used, and the order and prioritisation of testing. It also provides evidence for assessing the appropriateness of current treatment regimens and the feasibility of future modified regimens. Lessons learned during the survey should be drawn upon to strengthen sample referral systems and expand laboratory networks whilst striving towards establishing continuous surveillance systems based on routine DST.

A draft survey report should be developed, including background, objectives, methods, results and discussion sections. Service and policy implications should be thoroughly described in the report with a set of specific recommendations for action. Broader stakeholder consultations should follow to disseminate and discuss the key findings, secure political commitment and advocate for action. Priority interventions,

roles and responsibilities of key stakeholders and timelines should be identified during these consultations, and this information should be incorporated into the final survey report.

In addition to the comprehensive survey report, other materials targeting different audiences should be developed to disseminate survey findings and key messages. Examples include scientific manuscripts, a technical summary, a brief for policy-makers, media communication materials, a factsheet or infographics.

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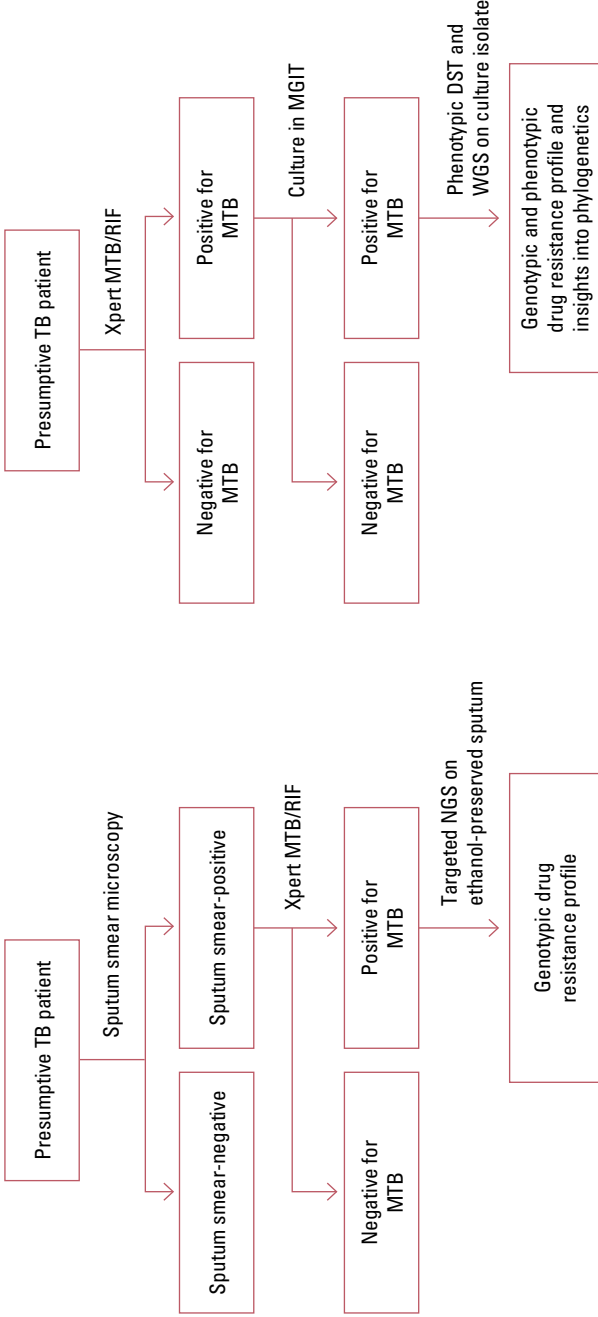
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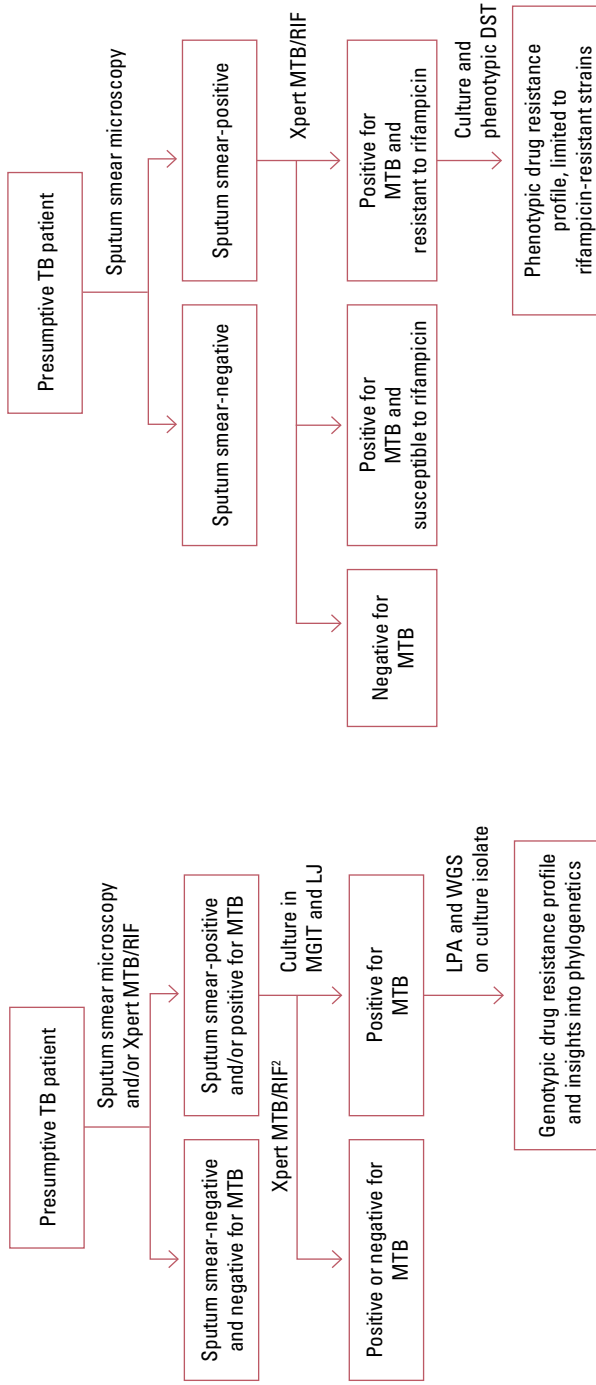
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Annex 1 – Examples of survey algorithm designs





Myanmar

See box in section 5.5 for further explanation.

DST - drug susceptibility testing; LJ - Löwenstein Jensen; MTB - *Mycobacterium tuberculosis* complex; NGS - next generation sequencing; WGS - whole genome sequencing

Togo

¹ Kayomo MK, et al. Targeted next-generation sequencing of sputum for diagnosis of drug-resistant TB: results of a national survey in Democratic Republic of the Congo. Sci Rep [Internet]. 2020 Dec 1;10(1):10786. Available from: <http://www.nature.com/articles/s41598-020-67479-4>

² For sputum smear-positive cases that did not have Xpert MTB/RIF performed initially

Annex 2 – Guide for developing a survey protocol

1 Principal investigator and human resources

- 1.1 Coordination/steering committee
- 1.2 Field team and other actors in the field
- 1.3 Technical assistance from topic experts

2 Introduction

- 2.1 Current TB epidemiological situation, including burden of TB, TB/HIV and drug-resistant TB
- 2.2 Structure of national TB programme and relevant health care providers
- 2.3 Central Reference Laboratory and laboratory network
- 2.4 Programmatic management of drug-resistant TB (PMDT)
- 2.5 Challenges for survey implementation in local context

3 Justification

4 Aim and objectives

- 4.1 Overall aim
- 4.2 Objectives

5 Materials and methods

- 5.1 Case definitions
- 5.2 Survey design
 - 5.2.1 Sampling frame
 - 5.2.2 Sampling strategy
 - 5.2.3 Sample size calculation
- 5.3 Intake period and survey implementation

6 Intake of patients

- 6.1 Inclusion criteria
- 6.2 Exclusion criteria

7 Sputum collection, storage, transportation and testing

- 7.1 Laboratory algorithm
- 7.2 Overview of the patient enrolment process
- 7.3 Collection and examination of sputum samples at health facilities
- 7.4 Storage and transport of sputum samples
 - 7.4.1 From health facilities to regional laboratories (if applicable)
 - 7.4.2 From regional laboratories to Central Reference Laboratory (if applicable)

- 7.4.3 From Central Reference Laboratory to SRL (if applicable)
- 7.5 Laboratory processing and testing of sputum samples
 - 7.5.1 Regional laboratories (if applicable)
 - 7.5.2 Central Reference Laboratory
 - 7.5.3 SRL (if applicable)
- 8 Capture, tracking and management (CTM) of samples and data**
 - 8.1 CTM at health facilities
 - 8.2 CTM at regional laboratories (if applicable)
 - 8.3 CTM at the Central Reference Laboratory
 - 8.4 CTM at the Supranational Reference laboratory (if applicable)
- 9 Electronic database and management of electronic records**
- 10 Analysis of survey data**
- 11 Survey planning**
 - 11.1 Training
 - 11.2 Survey preparedness assessment
 - 11.3 Pilot phase
- 12 Monitoring and evaluation**
 - 12.1 Quality assurance and quality control of laboratory procedures
 - 12.1.1 Microscopy (if applicable)
 - 12.1.2 Molecular methods (if applicable)
 - 12.1.3 Culture and phenotypic DST (if applicable)
 - 12.1.4 Next Generation Sequencing (if applicable)
 - 12.2 Monitoring of field activities
 - 12.2.1 Monitoring visits and remote monitoring
 - 12.2.2 Progress and quality reports
- 13 Survey governance**
- 14 Dissemination plan**
- 15 Ethical considerations**
- 16 Timeline**
- 17 Budget**
- 18 List of other survey documents**
- 19 References**
- Annexes**

Section	Points for consideration
1.1	Names and job titles of staff in the survey coordination team, including principal investigator, survey coordinator, data manager, epidemiologist/statistician, other.
1.2	Names and job titles of staff in the field team, including healthcare workers, laboratory technicians, staff of the national TB programme, regional TB focal points.
1.3	WHO, SRL, non-governmental organisations, academic organisations, other collaborators.
2.1	Country profile, including geography, population, TB and HIV epidemiological situation; data from previous drug resistance surveys if available; data from the previous cohort analyses (including case-finding and treatment outcome data) if available.
2.2	Information about the national TB programme, including strategy and operational design; mapping of all relevant health care providers not formally linked to the national TB programme (public, voluntary, private and corporate).
2.3	Description of the Central Reference Laboratory and the national laboratory network, detailing systems for internal quality control and external quality assessment and indicating the relationship with an SRL; description of any quality-assured non-programme laboratories willing to participate in surveillance activities.
2.4	Programmatic management of drug-resistant TB, including treatment regimens delivery of care to patients with rifampicin resistance or with Hr-TB.
3	Reasons for conducting a drug resistance survey, including any weaknesses in surveillance and programmatic management of drug-resistant TB and how this survey will address those.
4.1	General statement of intent about what the study aims to achieve overall.
4.2	Specific, achievable, realistic and time-constrained statements, that define measurable outcomes in light of the chosen survey design and the setting.
5.1	Relevant case definitions for the survey, including what is meant by bacteriologically confirmed TB, new cases and previously treated cases (and its subcategories).
5.2	Description of choice of sampling strategy (100% versus cluster sampling); formula and parameters used to calculate the sample size overall and by health facility.
5.3	Expected duration of patient enrolment, including an estimation of the duration of the intake period; whether enrolment will begin simultaneously in all health facilities, or whether a staggered design will be used.
6.1	Conditions that must be met in order to be enrolled in the survey.
6.2	Conditions that disqualify inclusion in the survey.
7.1	Brief overview of the survey laboratory algorithm (combination of laboratory tests that will be used for bacteriological confirmation of TB and DST, including the order of tests and the site where these tests will be performed). A flow-chart is recommended.
7.2	Brief introduction of the patient enrolment workflow following bacteriological confirmation of TB (age-appropriate informed consent or assent; HIV testing and counselling; patient interview; sample collection; assigning a unique survey identifier and registering the patient in the enrolment log).
7.3	Methods for initial bacteriological diagnosis of TB at health facilities; methods for collection and stabilisation of sputum samples for use in the survey.
7.4	Storage of samples before shipment; shipment schedule; transportation mode (courier, health facility vehicles, other); required packaging and transportation temperatures; expected transportation turnaround times; logistic aspects.
7.5	Detailed laboratory methods for DST at referral laboratories; biosafety precautions; methods for sample preparation, handling and storage (short- and long-term).

8.1	Data capture processes and description of forms used at this level to register patients enrolled into the survey and to track survey samples, for example the consent form, the case report form to classify cases according to previous anti-TB treatment history, the patient enrolment log, shipment forms, sample storage logs; how samples would be labelled; how to assign unique identification codes to health facilities, patients and samples; data collection and tracking forms purposively designed for the survey (included as annexes).
8.2 – 8.4	As per 8.1, according to the site, as well as procedures for feedback of test results to health facilities.
9	Database design; data entry; back-up of data; data validation and quality assurance; confidential management of records and forms during and after the survey; any additional documents providing a more detailed data management plan.
10	Interim and final data analysis plan; key outcomes to be analysed; statistical analyses to be performed.
11.1	Timing, location and content of training as well as participants and their roles and responsibilities. This section should refer to additional documents for details of the training packages.
11.2	Relevant checklists should be provided as annexes.
11.3	Timing and design of the pilot, including selection of health facilities.
12.1	Internal and external laboratory quality assurance and quality control processes, including reference to additional documents such as the quality plan as appropriate.
12.2.1	Responsibilities for internal and external monitoring activities; description of monitoring activities; the anticipated monitoring frequency and schedule. Relevant monitoring checklists and forms should be provided as annexes or within a separate monitoring plan.
12.2.2	Indicators to be used to inform the quality and progress of the survey during survey management meetings and trigger of corrective actions. The survey-specific list of quality and progress indicators should be provided as annexes or in the survey quality plan.
13	Survey management and survey team capabilities; staff roles and responsibilities; meeting schedules; communication and supervision plans. Additional documents can be referred to for more details regarding the survey organogram, job specifications and requirements, such as a communications plan or a responsibility delegation log.
14	Dissemination of results to key stakeholders and the scientific community, which may include meetings, reports, conference presentations and journal articles.
15	Age-appropriate informed consent or assent process; confidentiality principles; measures to ensure that patients receive appropriate care; review of protocol by relevant ethical committees.
16	Month-by-month timeline (a Gantt chart is recommended - see example below) for the planning and implementation of the survey, and analysis and dissemination of results.
17	Itemized budget including for consumables and laboratory supplies; human resources for the entire period of survey planning, implementation, analysis and dissemination of results; sample transport; survey monitoring; testing at SRL; technical assistance; other.
18	These are separated documents that may include standard operating procedures (SOPs); communications plan; risk management plan; quality management plan; survey monitoring plan; data management and analysis plans; policies for ownership, access to and re-use of survey specimens and data; financial and technical agreements, including material transfer agreements between laboratories. Some of the abovementioned content may be directly incorporated into the protocol (main text or annexes), rather than described in a separate document.
Annexes	Clusters included in the survey; laboratory algorithm flowchart; CTM chart; participant information sheet; consent/assent forms; data collection forms; checklists for preparedness assessments and monitoring survey implementation in health facilities and laboratories; responsibility delegation logs; quality and progress indicators.

Templates to guide the development of survey documents and tools are available from WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys*. Geneva: World Health Organization; 2021.

An example of a timeline:

	MONTH*																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Formation of the survey coordination team	■																								
Protocol development		■	■																						
Development of other survey documents		■	■																						
Ethics approval					■																				
Procurement and distribution of supplies						■	■																		
Database development and testing					■	■	■																		
Training of field staff						■	■	■																	
Pilot in several sites									■																
Launch of full survey in all sites										■															
Ongoing patient recruitment in all sites											■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Laboratory activities											■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Data management											■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Survey preparedness assessment and monitoring						■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Mid-term external monitoring mission												■													
Re-testing of specimens by SRL for external quality assurance																					■	■	■	■	■
Analysis and dissemination of results																								■	■

* The numbers should be replaced with the actual month and year, for example January 2021.

Annex 3 – Guide for survey participant information

The following information should be developed for each survey and provided in writing to eligible participants (and their legal representative or an impartial witness, where appropriate) as the first step of the process of informed consent or assent. The information must be written using lay language for a general audience. The information sheet should be age-appropriate. See also WHO's *Guidance for ensuring good clinical and data management practices for national TB surveys*. Geneva: World Health Organization; 2021.

Invitation Paragraph

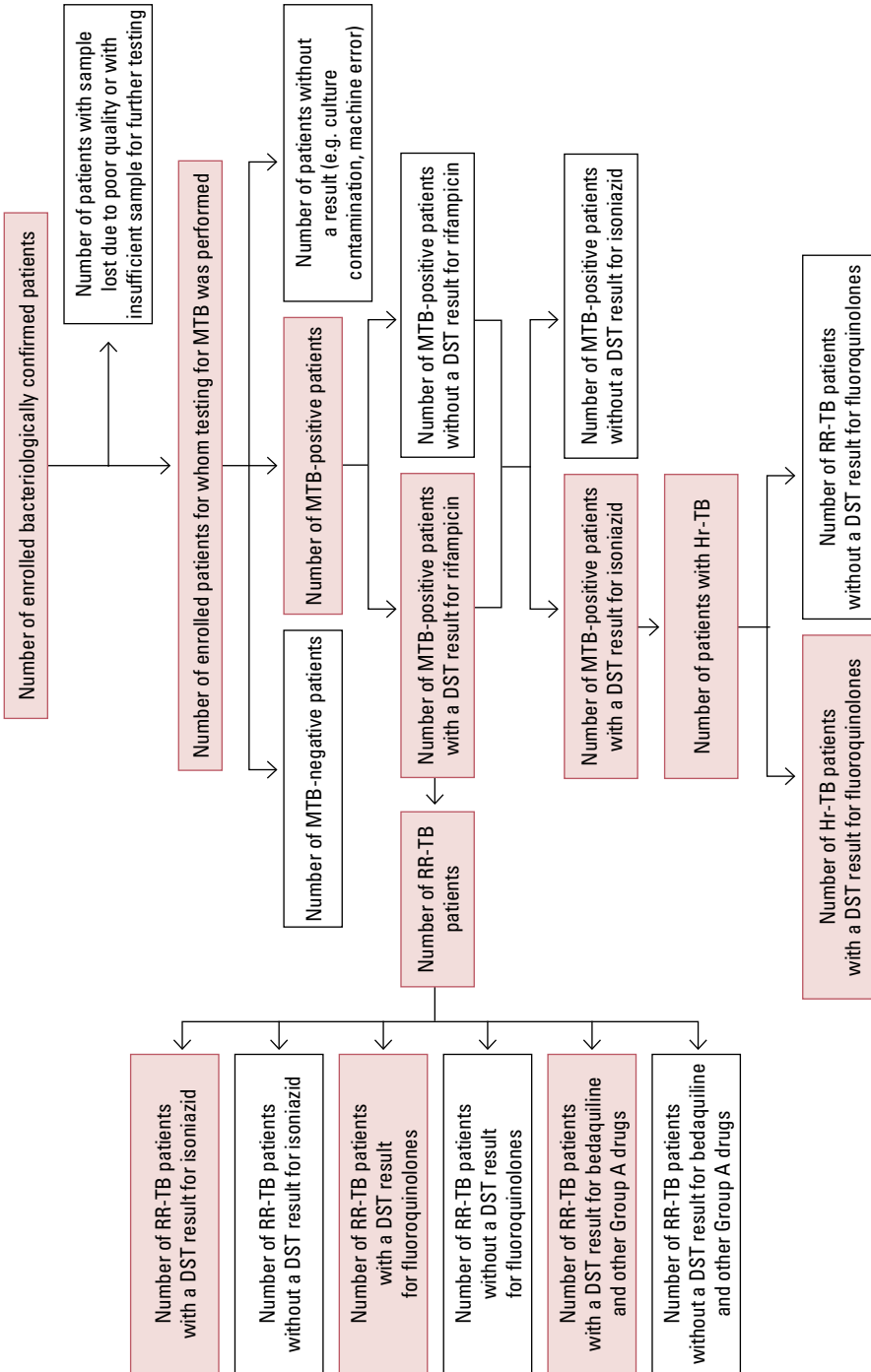
Part One - purpose of this survey and what will happen if participation is agreed

- Why do we need this study?
- What is the purpose of the study?
- Why have I been invited?
- Do I have to take part, and can I change my mind?
- What will happen if I take part?
- What will happen to my samples and any information collected?

Part Two - detailed information about how the survey will be conducted

- What are the possible disadvantages and risks of taking part?
- What are the possible benefits of taking part?
- What will happen to my information?
- Will my health care provider be contacted?
- Where will my samples be sent, and will my samples be kept confidential?
- What happens if I am found to have drug-resistant TB?
- What if there is a problem or I have a concern about this survey? (Who should I contact?)
- What will happen at the end of the survey?
 - What will happen to my samples?
 - What will happen to my information?
 - What will happen to my samples and information if I later decide I do not want to take part?
- Who is organizing and funding the survey?
- Who has reviewed the survey?
- Further information, clarifications and questions.
- Contact details of principal investigator and other relevant survey staff.

Annex 4 – Example of a flowchart of enrolled patients



DST - drug susceptibility testing; Hr-TB - rifampicin-susceptible, isoniazid-resistant tuberculosis; MTB - *Mycobacterium tuberculosis* complex; RR-TB - rifampicin-resistant tuberculosis. The flowchart should be modified to match the inclusion criteria and laboratory testing methods used. The numbers of patients in each box should be disaggregated by treatment history (new versus previously treated). Where available, numbers of patients with DST results should be reported for each drug individually. The selection of drugs considered in the laboratory algorithm for the survey depends on the availability of reliable DST and the capacity to perform the required assays.

Annex 5 – Tables for summarizing main results

Prevalence of resistance to rifampicin and/or isoniazid

	New patients		Previously treated patients	
	% (n/N)	95% CI*	% (n/N)	95% CI*
RR-TB ⁺				
Isoniazid-resistant TB				
Hr-TB				
MDR-TB				
Other [‡]				

Prevalence of resistance to other drugs among drug-resistant TB patients

	RR-TB		Hr-TB	
	% (n/N)	95% CI*	% (n/N)	95% CI*
Levofloxacin				
Moxifloxacin				
Any fluoroquinolone	++	++		
Bedaquiline				
Linezolid				
Any fluoroquinolone and at least one other Group A drug	**	**		
Other [‡]				

CI - confidence interval; Hr-TB - rifampicin-susceptible, isoniazid-resistant TB; MDR-TB - multidrug-resistant TB; n - number of patients with resistance to a given drug; N - number of patients for whom a DST result is available for a given drug; RR-TB - rifampicin-resistant TB, including MDR-TB

* Multiple imputation of missing DST results among bacteriologically confirmed pulmonary TB cases may be required (see section 7.2: Data analysis).

** The 95% confidence intervals should account for a clustered survey design, if relevant (see section 7.2: Data analysis).

++ This corresponds to the revised definition of pre-extensively drug-resistant (pre-XDR) TB from 2021.

** This corresponds to the revised definition of XDR-TB from 2021. The denominator should be restricted to patients for whom DST has been performed for all Group A drugs.

‡ The prevalence of resistance should be calculated for each additional individual drug for which DST results are available. Any other drugs tested among new and previously treated patients or among drug-resistant TB patients can be added to the above tables, including those of first-line or second-line regimens.

Number of patients with RR-TB

Treatment history

	New	Previously treated	Unknown	Total
RR-TB				
Rifampicin-susceptible TB				
Total				

HIV status

	HIV-positive	HIV-negative	Unknown	Total
RR-TB				
Rifampicin-susceptible TB				
Total				

Sex

	Male	Female	Unknown	Total
RR-TB				
Rifampicin-susceptible TB				
Total				

Age (years)

	0–4	5–14	15–24	25–34	35–44	45–55	55–64	≥65	Unknown	Total
RR-TB										
Rifampicin-susceptible TB										
Total										

Annex 6 – Template for survey budget

This template is provided for guidance and will require modification for each survey. The components will differ according to the survey design and needs of each country.

Item	Type of unit	Cost per unit	Number of units	Total
Human resources				
Principal investigator(s)				
Supervisor of laboratory activities				
Survey coordinator				
Database designer				
Data manager(s)				
Laboratory technician(s)				
Logistics staff (for example drivers, secretary)				
Subtotal				
Coordination meetings (central and peripheral levels)				
Per diem				
Transportation of participants				
Meeting room hire and catering				
Subtotal				
Training courses				
Per diem				
Transportation of participants				
Meeting room hire and catering				
Subtotal				
Data management system				
Software				
Servers and/or cloud hosting; back-up system				
Internet connection				
Subtotal				
Monitoring and supervision				
Per diem				
Transportation of supervisors to survey sites				
Subtotal				

Item	Type of unit	Cost per unit	Number of units	Total
Communication				
General (for example stationery, printing)				
Computer(s)				
Mobile phone credit				
Subtotal				
Laboratory				
Sputum containers				
Safety cabinet, if required				
Centrifuge, if required				
Reagents, Xpert cartridges, etc.				
Other (for example refrigerators; materials and reagents for storage of specimens)				
Subtotal				
Collection and domestic transport of specimens				
Transport containers and packaging				
Transport costs				
Subtotal				
Collection and international transport of specimens to SRL				
Transport containers and packaging				
Transport costs				
Subtotal				
SRL technical assistance				
Visits for survey planning and monitoring				
Laboratory proficiency testing costs				
Sample transport and retesting for external quality assurance of results				
Any other tests to be performed				
Subtotal				
Epidemiological technical assistance				
Visits for survey planning and monitoring				
Subtotal				
Finalization and dissemination of results				
Data cleaning and analysis				
Report writing and publication				
Dissemination meeting				
Subtotal				
				TOTAL

Annex 7 – Template for a case report form

Patient's survey identification number:

Health facility name: Health facility code:

Name of interviewer:

If already registered, patient's TB register number:

A. IDENTIFICATION OF THE PATIENT

1. Given name^a: Family name^a:

2. Date of interview: / / (Day/ Month/ Year)

3. Sex: Male Female

4. Date of birth: / / (Day/ Month/ Year) 5. Age: years

6. Date of sputum collection: sample 1 / / (Day/ Month/ Year)

sample 2 / / (Day/ Month/ Year)

Country-specific data (to be decided by the coordinating team), for example:

7. HIV-status: Negative Positive Unknown

[Additional questions relating to other possible risk factors as described in the protocol (see section 2.2)]

B. HISTORY GIVEN BY THE PATIENT

8. Previously treated for TB? No Yes Unknown

If 'No' to Question 8, go to Question 9^b. If 'Yes' to Question 8, go to Question 17.

9. For how long have you been sick?

10. Did you have the same symptoms prior to this episode?

No Yes Unknown

11. Did you have other symptoms of lung disease prior to this episode

(haemoptysis, chest pain, cough)? No Yes Unknown

12. Did you have sputum examinations prior to this episode?

No Yes Unknown

13. Did you ever take tuberculosis drugs for more than one month?

No Yes Unknown

14. If yes, what was the name?

15. Did you ever have injections for more than one month?

No Yes Unknown

16. Did the patient remember previous treatment for TB after these questions?

No Yes Unknown

C. MEDICAL RECORDS

17. After extensive checking through the medical files and other documents available in the health facility, have you discovered that the patient has been registered for tuberculosis treatment before?

No Yes Unknown

18. Previous TB registration number

D. FINAL DECISION

19. Patient has been previously treated for TB for more than a month:

No

Yes (answer to Question 8, 16 and/or 17 was 'Yes')

Unknown

20. If 'Yes' to Question 19, what was the most recent type of regimen received and the date of treatment initiation?

Drug-susceptible TB regimen: New Previously treated

Drug-resistant TB regimen: New Previously treated

Date: / / (Day/ Month/ Year)

21. If 'Yes' to Question 19, what was the outcome of previous treatment?

Cured

Treatment completed

Treatment failed

Lost to follow-up

Not evaluated

^a Depending on the country, it may be appropriate for the case report form to contain patient identifying information to ensure traceability of clinical records. Identifiable data must never be shared outside the programmatic and clinical teams and must be securely stored. Its inclusion in the case report form should be clearly justified as essential, and is subject to the approval of the relevant ethics committee.

^b Some patients may not immediately recall past treatment for TB or may not be aware that previous treatment was for TB. Questions 9-15 can be used by the investigator to assist the patient in recalling past treatment. Positive responses should prompt the investigator to follow up on questions to determine whether past treatment could have been for TB. For more information, see section 6.2.1: Case report form. Only the final decision of treatment history (Questions 18-19) needs to be entered into the electronic survey database.

Annex 8 – Storage of sputum samples and culture isolates

Scope

Leftover sputum samples, digested-decontaminated specimens and *M. tuberculosis* complex culture isolates should be systematically stored for the entire duration of the survey until a final and complete drug resistance profile is obtained. The storage conditions described in this document promote preservation of bacteria viability and maintain an intact genetic background over time.

Equipment and materials

- Biological Safety Cabinet, Class I or II, which must be certified annually
- Autoclave
- Freezer at -20°C and/or -80°C
- Sterile 2mL cryovials with external-thread screw cap
- Sterile disposable loops
- Sterile plastic pipettes
- Rack
- Separate waste containers (autoclavable) for pipettes and disposables
- Cryovial storage boxes

Reagents and solutions

- Middlebrook 7H9 broth, prepared according to manufacturer's instructions, and enriched with OADC or ADC
 - Sterile glycerol for molecular biology, $\geq 99.0\%$ (for example Sigma-Aldrich Cat N. G5516-500ml)
- As an alternative to OADC/ADC-enriched 7H9 medium plus glycerol (20%), sterile 10% skim milk medium can be used as storage media. This is prepared by mixing 100mL skim milk powder with 1L distilled water and autoclaving at 121°C for 15 minutes. It can be used to preserve *M. tuberculosis* complex isolates for 2 years at -20°C or for 10 years at -80°C .

Biosecurity and biosafety requirements

- Appropriate personal protective equipment
- Appropriate disinfectant (concentration and expiry date)
- Biological Safety Cabinet Class II with yearly certification
- Disposal of all contaminated material and cultures after autoclaving.

Short- and mid-term storage of culture isolates

Cultures on egg-based solid medium (for example Löwenstein Jensen medium) should be preferably stored at 2–8°C and may be maintained viable for up to one year. These cultures may also be kept at room temperature (maximum 20°C, in air-conditioned rooms if necessary) away from light, but media quality may deteriorate and viability may be affected.

To avoid multiple thawing-freezing cycles that will compromise the viability of the bacilli in the event of an unreliable power supply, successive subculture of isolates may be performed up to five times, at four-month intervals.

Storage of liquid cultures for more than one month is not recommended since media quality deteriorates rapidly and affects viability, while clumping in liquids makes the determination of bacterial concentration highly unpredictable. Moreover, liquid media are more prone to contamination.

Long-term storage of culture isolates

- The viability of the organisms declines much more rapidly at –20°C than at –80°C. Only 1% of bacteria are still viable after two years at –20°C compared with 100% at –80°C. It is thus crucial to store the heaviest bacterial load possible in order to compensate for the loss of viability.

Storing solid cultures

1. Prepare Middlebrook 7H9 medium with glycerol to a final concentration of 20% and autoclave. This can be stored at 4°C for up to six months.
2. Select colonies showing good, confluent and pure growth of *M. tuberculosis* complex on a Löwenstein Jensen slant within 10–15 days of first appearance. Older cultures will not provide reliable long-term viability.
3. Label cryovial tubes with the identification number of the isolate that can be linked to the unique survey identification number of the patient.
4. Using a sterile loop, carefully scrape as many colonies as possible from LJ or from agar medium without taking any culture medium. Suspend the colonies in a 2mL cryovial containing the 1.5mL sterile Middlebrook 7H9 medium with 20% glycerol prepared in step 1.
5. Store at –80°C for up to several decades (or at –20°C for several years) in cryovial storage boxes.
6. Record the location and isolate identifiers in a freezer storage logbook and survey database.

Storing liquid cultures

1. Select a well-grown, non-contaminated liquid culture (MGIT tube or OADC/ADC-enriched Middlebrook 7H9 broth) of each isolate that should be stored.
2. Vortex the tube for at least for one minute. Ensure the suspension is well dispensed and very turbid (greater than a 1.0 McFarland standard). Let the suspension stand undisturbed for 20 minutes.
3. Label cryovial tubes with the identification number of the isolate that can be linked to the unique survey identification number of the patient.
4. Add 0.3mL of sterile glycerol ($\geq 99.0\%$) to the tube.
5. Transfer 1.2mL liquid culture suspension to the labelled cryovial tube.
6. Store at -80°C for up to several decades (or at -20°C for several years) in cryovial storage boxes.
7. Record the location and isolate identifiers in the -freezer storage logbook and survey database

Storage of leftover sputum samples and digested-decontaminated sputum samples

Leftover sputum samples should be systematically stored either at -20°C or ideally at -80°C , without the addition of chemical preservatives, for the entire duration of the survey and until final DST results are obtained. This maintains the viability of the bacteria.

Similarly, the leftovers of digested-decontaminated sputum samples should be transferred into sterile cryovials and stored at -20°C or -80°C , to allow repeat testing in the event of culture contamination. In addition, both the sputum samples and digested-decontaminated sputum samples could serve as starting material for DNA extraction for subsequent targeted Next Generation Sequencing (NGS).

Waste management

Autoclave all solid and liquid cultures and other contaminated materials prior to disposal.

Annex 9 – Safe shipment of specimens

Part I - Shipping of culture isolates and sputum samples

An infectious substance is defined as any material containing a biological agent capable of causing disease in humans or animals. All infectious substances are designated as Dangerous Goods Class 6, Division 6.2. They are assigned a specific UN number (for example UN3373, UN2814) which determines the type of packaging, marking, labelling and documentation required for shipping.

Classification of *M. tuberculosis* complex-containing infectious substances

Category A: These agents are transported in a form that could cause permanent disability, or life-threatening or fatal disease in exposed but otherwise healthy humans or animals. *M. tuberculosis* complex cultures meet this definition. They are assigned the UN2814 number and shipped as “Infectious substance affecting humans”.

Category B: These agents are capable of causing infection in humans or animals, without meeting the criteria for Category A - the consequences of an infection are not considered severely disabling or life-threatening. *M. tuberculosis*-containing material in any form other than culture (for example sputum sample or sputum sediments) meets this definition. They are assigned the UN 3373 number and shipped as a “Biological substance”. An important exception to this classification is when *M. tuberculosis* complex cultures intended for diagnostic or clinical purposes are transported to the testing laboratory by ground transportation (for example road transportation from a peripheral laboratory to the Central Reference Laboratory). Under these circumstances, they may be classified as Category B according to the *European Agreement concerning the International Carriage of Dangerous Goods by Road (1)*.

Transport and packaging of *M. tuberculosis* complex-containing infectious substances

To ensure safe and expeditious transport, specific national and international regulations must be followed. Compliance with these shipment requirements is the responsibility of the shipper, who must be familiar with the regulations. Failure to comply may result in fines and other penalties.

Local ground transportation

This may be done by various means – by courier, health facility vehicles, other means of transport such as motorcycles, or “hand delivery” by health facility staff. All persons transporting specimens should be provided with training on biosafety and have spill kits accessible in case of accidents. All transporters should follow local regulations where applicable. The basic packaging system for local surface transport of all *M. tuberculosis* complex specimens consists of three layers:

- **Primary receptacle** – the specimen container, packaged with enough absorbent material to absorb all fluid in case of breakage. There is no maximum quantity of samples per package.
- **Secondary packaging** – a second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material must be used to absorb all fluid in case of breakage. For cold transportation conditions, ice or dry ice shall be placed outside the secondary receptacle. A leak-proof container should be used if regular ice is used.
- **Outer packaging** – secondary packaging is placed in an outer shipping packaging with suitable cushioning material. The outer packaging protects their contents from external influences, such as physical damage, during transit.

Air transportation

For domestic flights within the country, national civil aviation authorities apply national legislation. For international flights, IATA regulations should be followed (2).

Packaging of Category A (UN2814) infectious material (P620): UN 2814 infectious substances must be packaged in accordance with the packaging instruction P620 (IATA). An example of a primary receptacle suitable for shipping culture isolates is unbreakable 1.5 - 2mL screw-capped plastic tubes secured by Parafilm. The three-packaging layers must meet specific requirements. P620 compliant packaging material should be purchased only from manufacturers able to demonstrate compliance with these requirements and must be identified by a UN specification mark. For a detailed description of the P620 packaging material and an example of P620 packaging refer to WHO's *Guidance on regulations for the transport of infectious substances 2019–2020* (3). For air transport of Category A infectious agents different quantity limits apply depending on the aircraft. For shipments being carried in the cargo hold of passenger aircraft, no more than 50 mL or 50 g of Category A infectious substance per package is allowed. For shipments being carried on a cargo only aircraft, no more than 4 L or 4 kg of Category A infectious substance per package is allowed. *M. tuberculosis* culture isolates can be shipped at room temperature. Generally, training in IATA transportation regulations for packaging, labelling, and transport is required.

Packaging of Category B (UN3373) infectious material (P650): UN 3373 infectious substances must be packaged in accordance with the packaging instruction P650 (IATA), which provide a slightly more detailed set of triple packaging requirements than is the case for the basic triple packaging system described above for local ground transportation. It is generally feasible to source P650 compliant packaging materials from local manufacturers or suppliers. In this case, the manufacturers or suppliers should provide clear instructions to the user (shipper, sender or consignee) on how to correctly fill and close the package to ensure full compliance with P650. For a detailed description of the P650 packaging material and an example of P650 packaging refer to WHO's *Guidance on regulations for the transport of infectious substances 2019–2020* (3). For air transport of Category B infectious agents, the primary receptacle must not exceed 1L for liquids or the outer packaging mass limit for solids. The volume

shipped per package shall not exceed 4L or 4 kg. These quantities exclude ice, dry ice or liquid nitrogen that may be used to keep specimens cold. For sputum specimens, the addition of ice packs is generally sufficient to preserve the quality of the samples during the transport, provided that the turnaround time from package collection to its arrival to the referring laboratory does not exceed 5-7 days. Generally, training in IATA transportation regulations for packaging, labelling, and transport is required. For more information, see WHO's *Infectious substances shipping training* (4) and the *GLI Guide to TB specimen referral systems and integrated networks* (5).

References

1. European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR) and Protocol of Signature, done at Geneva on 30 September 1957 [Internet]. Available from: https://www.unece.org/index.php?id=50858&no_cache=1
2. International Air Transport Association (IATA). Dangerous Goods Regulations (DGR), 62nd edition. Montreal: IATA; 2021. Available from: <https://www.iata.org/en/publications/dgr/>
3. Guidance on regulations for the transport of infectious substances 2019–2020 [Internet]. Geneva: World Health Organization; 2019. Available from: <https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/>
4. Infectious substances shipping training - a course for shippers. Geneva: World Health Organization; 2015. Available from: https://www.who.int/ihr/i_s_shipping_training/en/
5. Global Laboratory Initiative. GLI Guide to TB specimen referral systems and integrated networks [Internet]. Geneva: GLI Working Group Secretariat; 2018. Available from: http://www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

Part II - Inactivation and shipment of non-infectious specimens for molecular testing

Scope

Instructions are provided for: i) heat-inactivation of *M. tuberculosis* complex culture isolates and sputum specimens, and ii) ethanol preservation of sputum specimens.

Note that for each isolate sent to the supporting SRL, the Central Reference Laboratory will be responsible for storing a back-up aliquot of the viable isolate at -80°C (refer to Annex 8), at least for the entire duration of the study and until the final drug resistance profile is obtained.

Equipment and materials

- Biological safety cabinet, class I or II, which must be certified annually
- Waterbath or thermomixer
- Pasteur pipettes

- 1.5 mL screw-cap vials
- 2 mL cryovial with external-thread screw cap
- Sterile loops)
- Solid medium (LJ or LJ supplemented with pyruvate) or liquid medium (MGIT or MBACT)
- Molecular grade water or sterile distilled water
- Ethanol 95-100%
- Parafilm
- Cryovial storage boxes
- Absorbent material (for example paper towels or absorbent bench paper)

Caution: before inactivation, the appropriate containment facilities and personal protective equipment should be used for specimen handling.

Heat-inactivation of culture isolates

1. *M. tuberculosis* complex isolated on solid media: using a sterile loop, carefully scrape as many colonies as possible without media and transfer to a 1.5mL to a screw-cap vial containing 1mL of distilled or molecular-grade water. *M. tuberculosis* complex isolated in liquid media: ensure the bacteria are well grown by leaving the tubes at 37°C for an additional 5-7 days from the first day of positivity to enrich the bacterial population in the culture media. The *M. tuberculosis* complex cell aggregates must be clearly visible in the transparent media. Transfer at least 1mL of the liquid culture into a 1.5 mL screw-cap vial.
2. Kill the cells by heating for 30 min at 95°C using either a thermal block or water bath and cool down to room temperature. The material is thus deemed non-infectious and can be handled outside the containment room (1,2).
3. Ensure that the samples are properly labelled with the identification number of the isolate that can be linked to the unique survey identification number of the patient.

Inactivation of bacteria in sputum samples

Bacterial cells in the sputum specimens can be inactivated using different methods: i) by heat-treating; or ii) by adding ethanol at 70% final concentration. The sputum samples must first be homogenized by letting them stand overnight at room temperature.

1. For heat-inactivation, transfer 1mL of homogenized liquefied sputum into a 2mL screw-cap vial and incubate for 30 min at 95°C using either a thermal block or water bath. Leave the vials to cool at room temperature.
2. For ethanol inactivation, transfer 0.3 mL of homogenized liquefied sputum into a 2 mL screw-cap vial containing 0.7 mL of 95-100% ethanol.
3. Tightly close the cap.
4. Ensure that the samples are properly labelled (unique survey identification number of the patient on the side of the vial).
5. Store at 2-8°C or room temperature until shipment.

Preparation for shipment

1. Sample packaging:
 - Place the screw-cap vials in a cryovial storage box and wrap each box with enough absorbent paper to absorb the liquid content of the package.
 - Seal the box with tape and place it into a leak-proof, sealable secondary plastic bag, with as much air removed as possible.
 - Place the box in an insulated expanded polystyrene (styrofoam) container of adequate size (optional).
 - The material is now ready for shipping.
2. Create an Excel file with the list of sample identification numbers ensuring linkage between the unique patient identification number and the sample or isolate identifier.
3. Confirm that the file matches the samples being shipped.
4. Email the completed Excel file to the supporting SRL at the time of the shipment and print a hard copy to accompany the shipment.

Shipping of inactivated samples

Shipping of heat-inactivated cultures or sputum samples

Heat-inactivated isolates can be shipped as non-dangerous goods/non-infectious material. To this end, a certification for non-dangerous goods as outlined in “Related Documents” below must be included. The declaration should be emailed to the supporting laboratory and a hard copy printed to accompany the shipment. The material can be shipped at room temperature using a regular courier service.

Shipping of ethanol-inactivated sputum samples

According to IATA regulations, ethanol is classified as a Dangerous Good belonging to Hazard Class 3 (UN1170) and, as such, transport must be regulated. However, for ethanol-containing solutions, exceptions may apply depending on the total amount (total volume) of material being shipped per package.

In particular, restricted quantity regulations exist for international shipping (referred to as IATA “Dangerous Goods in Excepted Quantities”). Ethanol concentrations between 10% and 80% fall into Packing Group III. For this group, the following quantities apply:

- the maximum net quantity per primary receptacle (such as individual tubes) is 30mL;
- the maximum net quantity per outer package (shipping box) is 1L.

In this case, an excepted quantity label needs to be applied on the package and the airway bill should specify “dangerous goods in excepted quantities”. A shipper’s declaration for dangerous goods is not needed.

When the material fulfils the so called “De Minimis Quantities” (IATA 2.6.10) as specified below, the package is no longer subject to IATA regulations and is not considered a dangerous good:

- the maximum net quantity per primary receptacle (such as screw-cap vial) is 1mL;
- the maximum net quantity per outer package (shipping box) is 100mL.

Ethanol-inactivated sputum specimens can be shipped at room temperature to the supporting laboratory using a regular courier service

Related documents – Shipper declaration for non-dangerous goods

[Shipper letterhead]

Date:

Destination:

Subject: Shipment of n° [] vials containing DNA (volume: maximum 1mL each)

To Whom It May Concern

CERTIFICATION FOR NON-DANGEROUS GOODS (NON-INFECTIOUS)

I hereby certify that the above-mentioned caption goods are non-hazardous materials for Air, Land, or Sea transportation in any nature. The consignment is fully described by proper shipping name and packed, marked and in proper condition for carriage by Air, Land or Sea. I further hereby certify that the content of the above-mentioned shipment is not pathogenic and is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. The material contained in this shipment is not infectious-contagious-toxic-corrosive-inflammable-explosive and it is not classified as Dangerous Goods under the current edition of IATA Dangerous Goods regulations and all applicable carriers and governmental regulations. No animal byproducts of any kind are present in this shipment.

This material has no commercial value and will be used for research only.

I also acknowledge that I may be liable for damages resulting from any misstatement or omission and I further agree that any air carriers involved in the carriage of this consignment may rely upon this certification.

Signature

References

1. Doig C, Seagar AL, Watt B, Forbes KJ. The efficacy of the heat killing of *Mycobacterium tuberculosis*. J Clin Pathol. 2002; 55:778-9.
2. Blackwood KS, Burdz TV, Turenne CY, Sharma MK, Kabani AM, Wolfe JN. Viability testing of material derived from *Mycobacterium tuberculosis* prior to removal from a containment level-III laboratory as part of a Laboratory Risk Assessment Program. BMC Infect Dis. 2005; 5:4.

Annex 10 – Template for assessment of survey preparedness and monitoring

This annex primarily addresses high-level governance aspects that should be assessed prior to the start of the survey. Selected items may also be monitored regularly during the survey (for more detailed guidance on field monitoring, see Annex 12). The form may be used by the survey coordination and field teams for self-assessment or for external monitoring purposes. Generic checklists are also available from WHO’s *Guidance for ensuring good clinical and data management practices for national TB surveys (1)*. Selected elements may be adapted to complement the tool presented here. This form should be adapted to the specific context of the survey and may be re-formatted to capture closed answers (“yes”, “no”, “not applicable”) in addition to narrative summaries of findings.

1. Survey management	Comments
Have the protocol, data collection tools and other survey documents been presented and discussed with the survey team (coordination team and field team)?	
Is there a human resources management plan and documentation detailing potential delegation of roles and responsibilities of staff? Document any deviations from the plan.	
Is there an itemized budget and financial management plan? Document any deviations from the plan.	
Is there a risk management plan? Document any deviations from the plan.	
Is there a plan addressing quality management aspects for the survey, including management and version control of survey documents as well as roles of staff in relation to assuring quality?	
Is there a system for tracking incidents or deviations from the protocol and informing the relevant ethics committee? Have any protocol deviations been identified? If yes, verify the reasons and the actions taken.	
Is there a communication plan detailing the strategies for communication between the survey coordination team, the field team, the health facilities, the Central Reference Laboratory and the SRL?	

Are work phones and/or mobile phone credit provided?	
Does the survey coordination team hold regular meetings? Review the minutes of recent meetings and actions thereof.	
Where applicable, are there documented policies for ownership, access to and re-use of samples and data?	
2. Informed consent/assent process	Comments
Is the design and contents of the participant information sheet and the assent/consent form adequate?	
Is the informed consent/assent procedure appropriate, including training? How will potential participants will be approached? Who will inform participants and obtain consent?	
3. Selection of health facilities	Comments
Has the selection of health facilities been conducted appropriately?	
How is the training of the health facilities and the laboratories organized? Are curricula, training materials and trainers available?	
Have any infrastructure gaps been identified, and is there a plan for addressing these?	
4. Supervision and monitoring	Comments
Is there a monitoring plan for site visits and remote assessments, including roles of different teams, quality indicators, checklists/guides?	
Are financial and human resources sufficient to perform the required monitoring tasks?	
Is there a system for regular reporting on monitoring activities (for instance regular meetings)?	
Are there training materials and activities for those conducting monitoring?	
5. Data management and analysis	Comments
Are data, samples and participants traceable to source documents (such as clinical notes and health facility registers)? Can data, samples and participants be correctly linked through a unique identifier?	
Are dedicated staff assigned for the data management, with clear roles and responsibilities?	

Is there a qualified and trained data manager?	
Is the electronic database appropriate? Are there built-in skip patterns, range and validation checks to ensure high-quality data? Have these been pilot tested to assess performance and validate the database structure?	
Is a clear data management plan documented and adhered to?	
Are the data protected by a password with access limited to a few authorized survey team members?	
Are there alternative solutions for electricity and internet shortage when using electronic systems? Is there a policy for back-up copies of electronic data?	
What quality control measures are in place to ensure data quality from collection until the preparation of the dataset for analysis?	
Do physical and electronic archives ensure secure and safe archiving conditions?	
6. Reporting and publication	Comments
Is there a detailed dissemination strategy, with sufficient allocation of resources?	
7. Additional comments / remarks	Comments
Is there any other problem identified and escalated by the survey team? Collect comments and feedback.	

References

1. Guidance for ensuring good clinical and data management practices for national TB surveys. Geneva: World Health Organization; 2021.

Annex 11 – Template for assessment of the preparedness and monitoring of the central reference laboratory

The core elements that need to be in place at the Central Reference Laboratory before the start of the drug resistance survey include the commitment to and, and capacity for, undertaking the survey, the existence of a quality assurance programme and a functional sample referral system. Assessors should be experienced staff from an SRL with a specific understanding of surveys. They should be familiar with and ideally should have contributed to the development of the country’s survey protocol and diagnostic algorithm.

This annex provides a minimum set of questions to support the assessors in the evaluation of the laboratory preparedness for each of the core elements described above. Selected items may also be monitored regularly during the survey. The assessors should check different sources of information during the review, including laboratory documents, direct observation of laboratory procedures and open questions to laboratory staff. In its current format, this tool captures a short fact-based answer for each item in the column “Assessment”, plus additional comments to help contextualise and interpret the findings. Generic checklists are also available from WHO’s *Guidance for ensuring good clinical and data management practices for national TB surveys (1)*. Selected elements may be adapted to complement the tool presented here. The form should be adapted to the specific context of the survey and may be re-formatted to capture closed answers (“yes”, “no”, “not applicable”) in addition to narrative summaries of findings.

1. Commitment to conducting the survey		
Question	Assessment	Comments, including source of information
If appropriate, has a Memorandum of Understanding (MoU) been signed between the national TB programme and the Central Reference Laboratory?		
Are the role and responsibilities of the Central Reference Laboratory clearly outlined in the MoU or survey protocol?		
If samples will be referred outside the country for further testing, is there a draft of the Material Transfer Agreement (MTA) available or under discussion?		

If samples will be referred outside the country for further testing, was it budgeted?		
If samples will be referred outside the country for further testing, were courier(s) selected for the shipment of the specimens to the SRL?		
2. Capacity to undertake the survey		
Question	Assessment	Comments, including source of information
Human resources capacity and competency		
Role and number of staff at the Central Reference Laboratory conducting sample testing (disaggregate by type of testing, for example molecular testing, culture and phenotypic testing)		
Role and number of staff at the Central Reference Laboratory performing other survey activities (disaggregated by type of activity, for example data management, quality assurance monitoring, training, on-site visits of peripheral laboratories)		
Role and number of staff that are newly hired for the survey versus those previously working at the Central Reference Laboratory		
Were competency assessments conducted for all the staff involved in the survey according to defined criteria?		
Was a training conducted to familiarise staff with the survey protocol?		
Are the staff involved in the survey knowledgeable regarding the survey diagnostic algorithm?		
Laboratory infrastructure and space		
Is the laboratory design and size adequate for the estimated additional workload?		
Is the laboratory infrastructure aligned with phenotypic/molecular DST requirements?		

Equipment availability		
Is the essential equipment for sample processing and testing available and sufficient in number to avoid disruption to routine activities?		
Is the laboratory equipment validated and regularly maintained?		
Is there a contingency plan to ensure continuous testing of survey samples in the event of an essential equipment breakdown?		
3. Quality assurance programme		
Question	Assessment	Comments, including source of information
Standard Operating Procedures (SOPs)		
Are SOPs covering all TB diagnostic technologies used in the survey algorithm available and consistent with international practice?		
Performance indicators		
Are quality indicators and performance measures monitored and evaluated for all TB tests? (see Annex 14)		
If performance indicators are below pre-set targets, have reasons been identified and corrective measures put in place?		
Internal quality controls		
Are internal quality controls in place for all TB tests included in the survey algorithm?		
Proficiency testing results and reports of on-site visits		
Does the laboratory participate in an international external quality assessment program to assess proficiency of phenotypic and molecular-based DST?		
Has the laboratory received an on-site visit by SRL staff within the last 12 months, and were any recommendations adequately addressed?		

Biosafety and safe working practices		
Does the laboratory undergo regular maintenance and is there an uninterrupted availability of general utilities (that is, stable, reliable, and adequate supply of electricity and water; stable communication lines)?		
Are biosafety and biosecurity requirements incorporated into SOPs according to international standards?		
Is there an adequate number of certified biosafety cabinets?		
If in place, is the air-handling system annually maintained including high-efficiency particulate air (HEPA) filter?		
Is safety equipment available for safely manipulating samples and culture isolates (for example personal protective equipment)?		
Consumables and reagents		
Is the forecasting of required laboratory supplies adequate? (including 10-15% additional repeated tests)		
Is there a contingency plan to ensure continuous testing of samples in case of unforeseen events affecting the procurement of laboratory supplies?		
Data management		
Is there an adequately trained data manager responsible for the collection, analysis and reporting of laboratory data generated during the survey?		
Is there a system in place that allows the real-time monitoring of survey progress (for example number of RR-TB cases diagnosed) and the performance of the diagnostic network (for example number of RR-TB tested for second-line drugs at the Central Reference Laboratory)?		

Is there a system in place that allows for a sample to be tracked from the referring laboratory to the referral laboratory for further testing, and for sharing of results?		
Are there procedures in place to ensure the security of laboratory data and the confidentiality of patient data?		
4. Functional sample referral system		
Question	Assessment	Comments, including source of information
Are SOPs covering the collection, storage and referral of samples available and consistent with international standards in terms of biosafety and biosecurity measures, packaging, and transportation?		
Do the required referral forms, registers, transport logs and tracing slips exist?		
Is there a system in place for monitoring key performance indicators for the sample referral system? For example, i) number of referred samples tested at the Central Reference Laboratory; ii) proportion of shipments that arrive within the specified transport time; iii) proportion of samples rejected due to inadequate or improper transport, packaging or documentation (disaggregated by referring site); iv) proportion of results that were transmitted to the referring laboratory within the specified turnaround time (TAT) after becoming available.		

References

1. Guidance for ensuring good clinical and data management practices for national TB surveys. Geneva: World Health Organization; 2021.

Annex 12 – Template for on-site assessment of the preparedness and monitoring of health facilities

This annex provides a form for assessing the preparedness or conducting a monitoring visit to a participating health facility. Some questions in the form are not applicable during the initial assessment and are only relevant to monitoring survey implementation, such as those relating to patient enrolment or the review of survey records. Generic checklists are also available from WHO’s *Guidance for ensuring good clinical and data management practices for national TB surveys (1)*. Selected elements may be adapted to complement the tool presented here. The form should be adapted to the specific context of the survey and may be reformatted to capture closed answers (“yes”, “no”, “not applicable”) in addition to narrative summaries of findings.

1. Training	Comments
How many survey focal points that attended training are still occupying their post and are currently in charge of survey procedures?	
Do new staff receive on-site survey training?	
Are back-up trained staff available who can undertake survey tasks if focal points are unavailable?	
2. Understanding of procedures and availability of SOPs	Comments
Can relevant staff correctly describe case definitions?	
Can relevant staff correctly describe the inclusion and exclusion criteria?	
Can relevant staff correctly describe the enrolment and laboratory workflow?	
Are SOPs available and accessible to relevant staff?	
3. Informed consent/assent process	Comments
Is the process acceptable as per WHO ethical principles?	
If children were enrolled, has the assent process been followed correctly (assent form signed by minor and consent form signed by parent/legal guardian)?	
If illiterate participants were enrolled, was a witness present and did he/she sign the consent form?	

Upon verification of a subset of forms, are forms signed or thumb-printed by all relevant parties? Was a witness signature obtained where applicable? Do participant details match those in the enrolment log? Was consent obtained by authorised trained staff? Do dates of participant and staff signatures match?	
4. Transport of samples	Comments
Can relevant staff correctly describe the processes to store, package and transport samples?	
Do laboratory technicians know the shipment addresses and relevant contact points at referral laboratories?	
Is a clear and adequate sample shipment schedule available and adhered to?	
Are sample transport arrangements well established and reliable?	
5. Equipment and power supply	Comments
Is necessary functional and reliable diagnostic equipment available on-site?	
Is the maintenance and calibration of equipment adequate and is the equipment housed appropriately (for example ventilation, temperature, other requirements)?	
Are functional and reliable cold-chain equipment available on-site (where applicable)?	
What are the provisions to cope with power cuts?	
6. Inventory	Comments
Have there been any stock-out of reagents or consumables since the start of the survey? This includes those required for sputum sample collection, laboratory testing for TB and HIV, preservation and transportation of samples.	
Are the required survey forms and registers available, and in use on-site (for example consent forms, case report forms, other)?	
7. Communications plan and strategy	Comments
Are communication channels in place between health facilities and regional and central survey focal points?	
Do staff know who to contact in case of concerns or questions?	
Is there a clear task delegation log in the event of the absence of the survey focal points?	
Are work phones and mobile phone credit available?	

8. Enrolment	New patients	Previously treated patients	Total
Number of bacteriologically confirmed pulmonary TB patients that have been eligible for enrolment since survey start date, according to routine registers			
Number of patients enrolled in the survey			
9. Feedback of laboratory results	Comments		
Is there timely feedback of laboratory results to the health facility and to the patient from referral laboratories?			
10. Inspection of registers and forms	Comments		
Is the record-keeping adequate and up-to-date in routine and survey registers?			
Is the identification of survey participants adequate in all relevant forms and registers, and is there consistency when cross-referenced?			
Are the reasons for missed enrolment systematically documented?			
Is the record-keeping of laboratory results adequate?			
Is there an appropriate filing system for survey forms and registers, in agreement with the survey protocol?			
On a subset of randomly selected survey patients, are the data complete, accurate and consistent (for example through in-depth inspection of consent forms, case report forms, test results, shipment forms)?			
11. Classification of patients by treatment history	Comments		
Are RR-TB patients re-interviewed to ensure correct classification? Is there a good level of agreement between the two interviews?			
12. Additional comments / remarks			

References

1. Guidance for ensuring good clinical and data management practices for national TB surveys. Geneva: World Health Organization; 2021.

Annex 13 – Template for remote monitoring of health facilities

This annex provides a form for systematically conducting remote monitoring of performance and progress of health facilities from the central or regional level by phone. The form should be adapted to the specific context of the survey.

Remote Monitoring Form

Date: / /	Name of person conducting the monitoring:
Name of health facility:	

	New	Previously treated	Total
Number of bacteriologically confirmed pulmonary TB patients that have been eligible for enrolment since survey start date, according to routine registers			
Number of patients enrolled in the survey			

If applicable, main reasons for missed enrolment: _____

Additional comments on any concerns raised which may include (but is not limited to): supplies; downtime of diagnostic equipment; staff availability, training and turn-over; sample transport. It is assumed that all people with presumptive pulmonary TB are tested bacteriologically to obtain confirmation of pulmonary TB.

Where appropriate, refresher training can be provided on key concepts such as case definitions, inclusion and exclusion criteria, or any other aspects.

Annex 14 – Examples of quality and progress indicators

The following is a list of indicators related to the progress and quality of the survey that should be monitored at least monthly. These indicators could be presented during regular meetings by the survey coordination team to guide decision-making. Most can be obtained from the electronic survey database if this has been appropriately designed and is kept up-to-date. The indicator list should be adapted to the specific context, particularly the survey laboratory algorithm. Not all indicators may be relevant.

Importantly, it should be noted that these indicators capture progress of the survey in terms of meeting its planned objectives and achieving its expected outcomes. The indicators are therefore mostly considered at the patient level, rather than by each individual laboratory test performed. Some patients may have the same test performed multiple times. Therefore, to monitoring laboratory performance, both routinely as well as during the survey, these indicators should be adapted to capture information about each individual test performed where appropriate. Further information can be found in the Global Laboratory Initiative (GLI) *Practical guide to TB laboratory strengthening* (1), the *Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing* (2) and the upcoming guide from WHO and FIND on *Practical considerations for implementing next-generation sequencing for drug resistance surveillance in national TB programmes* (3).

Testing dates should be systematically recorded, regardless of the availability of test results, because the latter may not be available for days or weeks, depending on the method considered. In contrast, to monitor testing outcomes, the number of patients with a final result is a better indicator. The dates of testing and final result are usually the same for molecular tests (Xpert MTB/RIF, Xpert Ultra, Truenat MTB-RIF Dx and LPA).

Notes: For each group of indicators, required indicators are listed first, followed by additional desirable indicators. The survey coordination team should define acceptable thresholds or TAT for relevant indicators before the start of the survey, guided by technical assistance from topic experts as needed. Deviations from the thresholds or TATs should trigger targeted action to improve performance and/or quality. It should be noted that this is a rolling report. All numerators and denominators should include all cases since the start of the patient enrolment period.

Indicator	Measure	Data source	Requirement
1. Progress of enrolment			
Proportion of eligible bacteriologically confirmed pulmonary TB patients in routine registers at health facilities that were enrolled in the survey ^{1,2}	Numerator: Number of enrolled patients. Denominator: Total number of eligible bacteriologically confirmed pulmonary TB patients in routine registers of the health facility	Numerator: Survey database cross-validated against monitoring forms (remote or site visit) Denominator: Monitoring forms	Required
Proportion of expected patients based on routine TB surveillance data that were enrolled in the survey ^{1,2}	Numerator: Number of enrolled patients Denominator: Total number of bacteriologically confirmed pulmonary TB patients notified to national TB programme for the same period in the same or previous comparable year(s)	Numerator: Survey database Denominator: Routine surveillance data	Desirable
2. Completeness of clinical and demographic data			
Proportion of enrolled patients for whom the final treatment history classification is missing ²	Numerator: Number of enrolled patients for whom the final treatment history classification is unknown Denominator: Total number of enrolled patients	Numerator: Survey database Denominator: Survey database	Required
Proportion of enrolled patients for whom data are missing for a key clinical or demographic variable ²	Numerator: Number of enrolled patients with missing data for a given variable (for example age, sex, HIV status) Denominator: Total number of enrolled patients Proportions should be calculated separately for each key variable	Numerator: Survey database Denominator: Survey database	Required
3. Turnaround time (tat) for sample transport and processing			
TAT from collection of samples at health facilities to arrival of samples at Central Reference Laboratory ²	Histogram showing days from date of sample collection to date of sample arrival at Central Reference Laboratory. The mean and median time should be shown. Table showing the cumulative percentage of samples arriving at day 0, 1, 2, etc. after sample collection.	Histogram: Survey database Cumulative percentage table: Survey database	Required

Indicator	Measure	Data source	Requirement
TAT from collection of samples at health facilities to testing of samples at the Central Reference Laboratory ²	Histogram showing days from date of sample collection at peripheral health facilities to date of sample testing at the Central Reference Laboratory. Histograms are shown separately for each test (for example MTB/RIF Xpert; LPA; initial culture inoculation in solid or liquid media; other). The mean and median time should be shown. Table showing the cumulative percentage of samples being processed at day 0, 1, 2, etc. after sample collection.	Histogram: Survey database Cumulative percentage table: Survey database	Required
TAT from collection of samples at health facilities to shipment of samples to the Central Reference Laboratory ²	Histogram and Table as above.	Histogram: Survey database Cumulative percentage table: Survey database	Desirable
4. Processing of samples at the central reference laboratory			
Proportion of total samples received at the Central Reference Laboratory that were rejected ²	Numerator: Number of sputum samples rejected on arrival Denominator: Number of samples received at the Central Reference Laboratory	Numerator: Survey database Denominator: Survey database	Required
Proportion of enrolled patients from whom samples were received at the Central Reference Laboratory which were tested ¹	Numerator: Number of enrolled patients with a documented testing date (for example Xpert MTB/RIF, LPA, inoculation date in culture media and for phenotypic DST) Denominator: Number of enrolled patients with date of sample receipt at the Central Reference Laboratory and who are eligible for the test, according to the survey algorithm. Proportions should be calculated separately for each test type.	Numerator: Survey database Denominator: Survey database	Required

Indicator	Measure	Data source	Requirement
5. Completeness and availability of testing results			
Proportion of enrolled patients with either no result, an invalid result, or an error for Xpert MTB/RIF or Ultra ^{1,3}	<p>Numerator: Number of enrolled patients with either no result, an invalid result or an error.</p> <p>Denominator: Number of enrolled patients with a testing date for Xpert.</p> <p>Proportions should be calculated separately for each classification.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required
Proportion of enrolled patients with <i>M. tuberculosis</i> complex detected at trace levels by Xpert Ultra ^{1,3}	<p>Numerator: Number of enrolled patients with MTB detected at trace levels.</p> <p>Denominator: Number of enrolled patients with a testing date for Xpert Ultra.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required
Proportion of enrolled patients with an invalid result for Truenat MTB or MTB Plus, and an indeterminate or error result for Truenat MTB-RIF Dx ^{1,3}	<p>Numerator: Number of enrolled patients with either an invalid result, an indeterminate result or an error.</p> <p>Denominator: Number of enrolled patients with a testing date for Truenat.</p> <p>Proportions should be calculated separately for each classification.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required
Proportion of enrolled patients with no interpretable result for LPA ^{1,3}	<p>Numerator: Number of enrolled patients with no interpretable LPA result.</p> <p>Denominator: Number of enrolled patients with a testing date for LPA.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required
Proportion of enrolled patients with either no culture growth or a contaminated culture result ^{1,3}	<p>Numerator: Number of enrolled patients with either no growth or a contaminated culture result.</p> <p>Denominator: Number of enrolled patients with a date for final culture result</p> <p>Proportions should be calculated separately for each classification.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required

Indicator	Measure	Data source	Requirement
Proportion of enrolled patients with contaminated or uninterpretable phenotypic DST due to lack of growth of control (drug-free) tubes/plates ^{1,3}	<p>Numerator: Number of enrolled patients with either a contaminated or uninterpretable phenotypic DST result.</p> <p>Denominator: Number of enrolled patients with a date for final phenotypic DST results.</p> <p>Proportions should be calculated separately for each classification.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	Required
Proportion of enrolled patients for whom NGS failed quality control ^{1,3}	<p>Numerator: Number of enrolled patients that failed NGS quality control criteria at any stage of the process.</p> <p>Denominator: Number of enrolled patients with a result date for NGS.</p> <p>Proportions should be calculated separately for each stage of protocol where failure occurred.</p>	<p>Numerator: Survey database and laboratory registers</p> <p>Denominator: Survey database and laboratory registers</p>	Required
Proportion of enrolled patients with no result or an invalid result from NGS ^{1,3}	<p>Numerator: Number of enrolled patients with either no result or an invalid NGS result.</p> <p>Denominator: Number of enrolled patients with a result date for NGS.</p> <p>Proportions should be calculated separately for each drug.</p>	<p>Numerator: Survey database</p> <p>Denominator: Survey database</p>	
6. Agreement of test results			
Cross-tabulation of test results from different tests ^{1,3}	<p>Grid showing count of patients in each outcome result combination from two or more tests.</p>	<p>Grid: Survey database</p>	Required

Indicator	Measure	Data source	Requirement
7. Tat for reporting of critical or final test results to health facilities			
TAT for reporting of a critical or final testing result from the Central Reference Laboratory to referring facilities ¹	Histogram showing days between obtaining test result at Central Reference Laboratories to reporting to the referring facility. Histograms are shown separately for the various test outcomes depending on the report timing requirements. Table showing the cumulative percentage of test results being reported at day 0, 1, 2, etc. from the date of the test result.	Histogram: Survey database Cumulative percentage table: Survey database	Required

¹ The indicator should be calculated separately for new and previously treated patients in the survey.

² The indicator should be calculated for each cluster (cluster sampling) or health facility (exhaustive sampling of all health facilities), and overall.

³ The indicator should be calculated per patient (for monitoring survey progress) and/or per individual test (for monitoring laboratory performance) as appropriate.

References

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2. Global Laboratory Initiative. Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing (“Xpert QA Guide”) [Internet]. Geneva: GLI Working Group Secretariat; 2019. Available from: <http://www.stoptb.org/wg/gli/assets/documents/Xpert-QA-guide-2019.pdf>
3. Practical considerations for implementing next-generation sequencing for drug resistance surveillance in national TB programmes. Geneva. World Health Organisation and FIND; in press.



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